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SORPTION OF ORGANIC SUBSTANCES BY ION EXCHANGERS OF VARIOUS NATURE

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Abstract - Sorption of biologically active substances by ion exchangers, characteristic features of such substances and their low stability levels are discussed. Main principles of choosing ion exchangers for isolation and purification of organic substances are developed.

Investigation of ion exchange with respect to organic substances revealed specific relationships in their sorption and indicated a necessity of synthesizing special sorbents for isolation, purification and separation of organic substances, most of all biologically active substances.

The main products isolated and purified with ion exchangers are biologically active substances, including antibiotics, semiproducts for their production, amino acids, peptides, alkaloids and enzymes. Natural products are usually polybasic. They are weak bases and acids. A great number of physiologically active substances belongs to the class of amphoteric compounds.

Instability in solutions and inactivation under the effect of various factors are the most significant critical features practically pertinent to all the objects. This imposes definite limitations on the methods of their production.

On the one hand, the sorbates greatly differ in their structure size and electrochemical properties and, on the other hand, they have a common feature, i.e. their susceptibility to the environmental factors inducing irreversible changes in the structure of the sorbates and the loss of their therapeutic activity.

Such properties require specific approaches to the problem of choosing ion exchangers and conditions for isolation and purification of the organic substances with them.

The statics and kinetics of ion exchange processes with respect to organic substances have been studied by us for many years and the following specific relationships were shown:

(1) The sorbent - solution system may be in 2 equilibrium states, i.e. true and false,

(2) the sorbent counterions induce catalytic transformation of organic counterions,

(3) various mechanisms are used simultaneously for sorption of organic substances by ion exchangers.

At first, the approaches to the choice of ion exchangers for sorption of organic ions are discussed. These approaches are closely connected with the structure and properties of organic ions and ion exchangers. During the usual process of ion exchange a thermodynamic equilibrium state is established in the system in spite of changes in the properties of the ion exchanger. However, under definite conditions, when organic ions are involved in the process of ion exchange, changes in the physical properties of the ion exchanger are so significant that a false equilibrium state is established which is a qualitatively new one. A distinctive feature of such a condition is availability of an infinitely large number of false equilibrium states for the same system and gradual transition to the true state.

When the equilibrium state is true, the counterions of the ion exchanger are uniformly distributed in the particles, while in the state of false equilibrium the distribution of the counterions of various types is not uniform.

After completion of organic substance sorption from a solution, the concentration of this substance in the peripheral layers of the sorbent is much higher than that in the center of the bead.

Two types of the terminal states in the process of methylene blue sorption by a carboxylic cation exchanger and the dye ion distribution in the particle after the process completion are presented in Fig.1.

There exists a concentration profile of counterions in the particles of ion exchangers. Such a profile changes under the effect of various factors, i.e. temperature, the particle size, initial counterion form and crosslinking, concentration of the competing ions in solutions, concentration of organic solvents in aqueous solutions, etc. (Refs 1-4).

The study on the kinetics of ion exchange with respect to organic ions showed that, when the equilibrium is established in the system, the initial diffusion coefficients of the organic ions, i.e. those calculated according to the gel kinetics mechanism, change insignificantly, while the initial content of the organic ions in the ion exchanger increases.

Nevertheless, when the false equilibrium state is established in the system, the initial diffusion coefficients of the organic ions markedly decrease with an increase in the level of the organic ions in the ion exchanger and the activation energy of diffusion markedly increases (Refs 5,6). Decrease in the diffusion rate during the ion exchange process is due to an increase in the rigidity of the ion exchanger layers mainly containing the organic counterions, which is shown in the study on the other properties of the ion exchanger, the water sorption isotherm and the specific volume of the sorbent containing different amounts of the organic counterions (Ref.7).

Both true and false equilibrium states are used in development of sorption methods for industrial production of organic substances. For concentration and purification of substances isolated from fermentation broths it is advisable to use ion exchangers providing replacement of all inorganic ions by the organic ones. Figure 2 shows the results of antibiotic sorption by various carboxylic cation exchangers. When the concentration of the sodium ions in the solution is low, i.e. less than 0.3 M, the antibiotic is sorbed by the low crosslinked cation exchanger with practically completed replacement of the sodium ions by the antibiotic ions (true equilibrium state). When a more highly crosslinked ion exchanger of the same type is used, the antibiotic level in the ion exchanger appears to be much lower (false equilibrium state). This indicates that in the first case the filtrate obtained by means of desorption has a higher concentration of the antibiotic and a low concentration of the accompanying ions.

The sorption rate of the main product by an ion exchanger is one of the most important characteristics defining its usefulness for the sorption of biologically active substances. As a rule, the sorption rate of organic ions is limited by diffusion inside the particles of ion exchangers. The larger the size and the higher the charge of the diffusing ion and the higher the crosslinking of the ion exchanger, the lower are the sorption and diffusion rates. It is demonstrated that the initial coefficients of the inner diffusion in the process of ion exchange sorption of organic ions are by 2-3 orders of magnitude lower than the respective coefficients for inorganic ions (Ref.8).

In the synthesis of ion exchangers various means are used for increasing the sorption rate with respect to organic ions. The synthesis of ion exchangers with real pores in the ion exchanger matrix is aimed to increase the sorption rate of organic ions and improve the technological characteristics of the sorbent. Usually, for formation of the pore structure of ion exchangers, their synthesis is performed in the presence of various neutral solvents (Refs 9,10). Figure 3 presents the initial coefficients of the inner diffusion of streptomycin in the carboxylic cation exchanger synthesized by the routine methods (gel cation exchanger) and by a method using neutral solvents (modified cation exchanger). When the gel cation exchanger is used, the diffusion coefficient of the antibiotic decreases with an increase in the crosslinking of the cation exchanger.

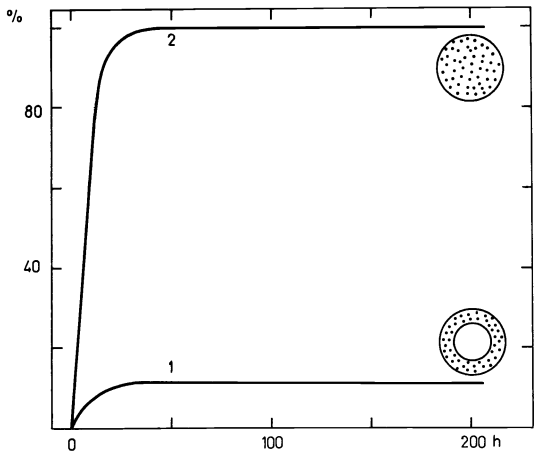


Fig. 1

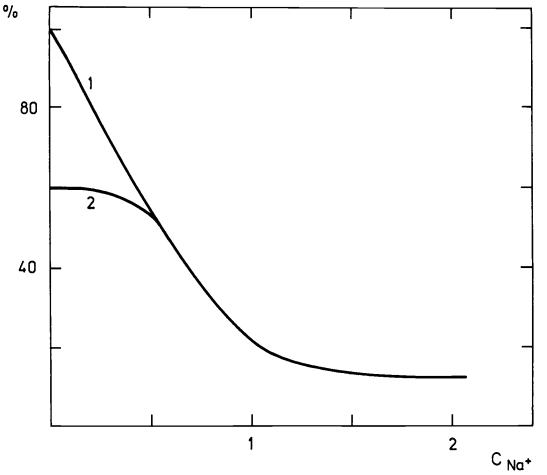


Fig. 2

Fig.1. Sorption of methylene blue by carboxylic cation exchanger KB-4 in Na form: (1) KB-4-10; (2) KB-4-2. Sorption given in % of the sorbed methylene blue amount to the resin static exchange capacity. Microphotographs of granule slices of cation exchangers KB-1 after methylene blue sorption (20x).

Fig.2. Sorption of streptomycin by cation exchanger KB-2 in Na-form in the presence of sodium ions (C_{Na^+}, mol/l): (1) KB-2-2,5; (2) KB-2-8. Sorption is given in % of the sorbed streptomycin amount to the resin static exchange capacity.

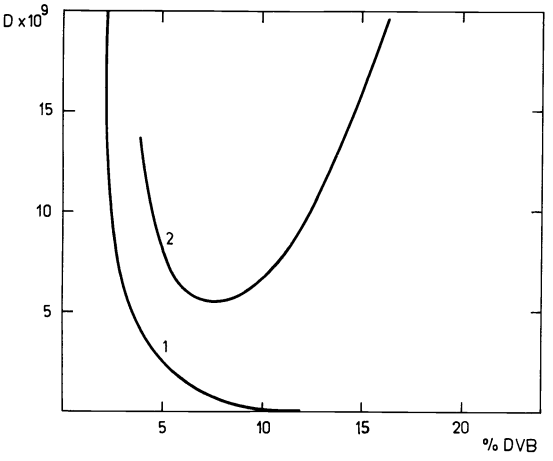


Fig. 3

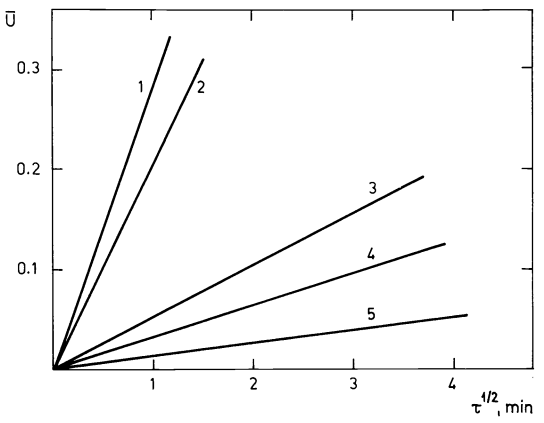


Fig. 4

Fig.3. Initial diffusion coefficients, D(cm^2s^{-1}), of streptomycin in the antibiotic sorption by modified carboxylic cation exchangers in Na-form and gel cation exchangers of various crosslinking levels: (1) gel cation exchangers, (2) modified cation exchangers.

Fig.4. Sorption of streptomycin into Na-form of carboxylic cation exchangers (10 per cent DVB) of various monomeric composition. $\bar{U}$ is the ratio of the sorbed streptomycin amount to the resin static exchange capacity. (1) Maleic acid, (2) fumaric acid, (3) itaconic acid, (4) acrylic acid, (5) methacrylic acid.

The rate of the antibiotic sorption by the modified cation exchanger is higher than that by the gel one (Ref.11). With an increase in the cross-linking of the modified ion exchangers there is observed a decrease in the diffusion coefficient. The exchange rate on cation exchangers with cross-linking of at least 8 per cent DVB markedly increases, which is due to formation of the pore structure of the ion exchanger. The favourable kinetic characteristics of the modified ion exchangers provide sorption and desorption of biologically active substances and regeneration of the ion exchanger in shorter periods. The other very important advantage of such ion exchangers is their osmotic stability (Ref.12). The volume of such carboxylic ion exchangers changes slightly on their transformation from the salt to the hydrogen form and vice versa.

The kinetic and electrochemical properties of ion exchangers depend on the nature of acids or bases used in their synthesis (Ref.13). The changes in the initial coefficients of inner diffusion of organic ions after their sorption by cation exchangers synthesized with the use of various monomer acids are presented in Fig.4. The use of maleic acid provides a significant improvement of the sorbent kinetic properties. On the other hand, such cation exchangers are capable of dissociation at higher pH as compared to the cation exchangers synthesized with the use of acrylic acid (pK_o = 7.0 and 5.5 for the cation exchangers synthesized with the use of maleic and acrylic acids respectively). Therefore, with the use of various carboxylic acids it is possible to prepare ion exchangers differing in their electrochemical properties.

Selectivity of the sorption of organic hydrophilic substances by synthetic ion exchangers is usually low. Antibiotics include a group of polyvalent bases, such as kanamycin, gentamycin, monomycin, sisomycin and others. The selectivity constants (antibiotic - sodium) of such substances on carboxylic cation exchangers of type KB-2 are 0.5-1.0 (Ref.14). Subsequently, for preparation of concentrated filtrates of the substances having a hydrophilic nature special procedures for "enrichment" of ion exchangers with organic substances were developed by us. When the cation exchanger and solution from which the product is isolated, e.g. fermentation broth filtrate, are in the state of equilibrium, a solution containing not only this product but also a complex forming agent is added to the cation exchanger (Ref.15). Such washing results in an additional sorption of the organic substance and intensive elimination of both the organic admixtures and the inorganic ions from the ion exchanger (Fig.5).

Another highly effective purification procedure may be used for sorption of enzymes with highly swelling resins. Thus, cation exchanger KB-2-0.5 was used for sorption of penicillinamidase in the form of a cation. For selective desorption potassium benzylpenicillin, an enzyme substrate was used. The procedure is based on a specific binding of the enzyme electroneutral form by the benzylpenicillin anion followed by formation of the Michaelis complex and its liberation from the ion exchanger. The use of the substrate as a selective desorbent provided a 5 fold increase in the enzyme purity as compared to the use of the phosphate buffer.

In industrial production of antibiotics with the use of ion exchange a rapid method is required for determination of the counterion composition of the ion exchanger after completion of antibiotic sorption from fermentation broth filtrates. The concentrations of the main counterions in an ion exchanger may be calculated using the concentrations of the respective ions in the filtrate and the selectivity constants for separate two ions (Refs 16-18).

The characteristic features of organic ions exchange connected with establishment of false equilibria are very often used for separation of the ions by their size (ion sieves). Thus, highly crosslinked ion exchangers are used for removal of inorganic ions, first of all sodium and potassium ions from solutions containing large organic ions. All the organic ions practically remain in solution (Refs 18-19). At the same time small organic ions may be separated from larger ions of organic admixtures having the same charge (Ref.18). Organic substances unstable in solutions also may be destructed in the sorbed state under the effect of H or OH counterions of the ion exchanger.

A study of stability of various biologically active substances, such as antibiotics, their semiproducts and enzymes in the sorbed state and in solution showed that at an equal acidity the destruction rate and the activation energy of the reaction in water and in exchangers are rather close. This allows to predict the sorption conditions providing the product stability in the sorbed state (Ref.20).

An indicator method of direct determination of the acidity function was developed for estimation of the catalytic activity of the hydrogen ions in a strong-acid resin with sulfonic groups (Ref.20). The acidity function of the ion exchanger (Ho) was calculated in the same way as that of the acid aqueous solutions according to the following equation:

$$\bar{H}_0 = p\bar{K}_a - \log(\bar{C}_{BH^+}/\bar{C}_{B0})$$

where $\bar{C}_{B0}$ and $\bar{C}_{BH^+}$ are the indicator concentrations in the ion exchanger in the nonionized and ionized forms, $\bar{K}_a$ is the ionization acid constant of the base indicator in the ion exchanger phase (concentrations are given in mol/l).

Since the indicator ionization constant in the resin phase may differ from that in solution (Refs 21-24), direct determination of K_a is necessary. The acid constant of the indicator ionization in the ion exchanger phase may be estimated directly using the method of spectrophotometry. The other method designated by us as an "ion exchange" method may be used for determination of the ionization constants of weak electrolytes in the ion exchanger phase. This method is based on the use of equations establishing relations between the sorption and electrochemical constants of the sorbates (Ref.22).

The indicator method may be used not only for the determination of the acidity function, but also for the estimation of pH, when the hydrogen ion concentration is 10^{-10} - 10^{-14} M.(Ref.23). The indicator method of estimation of the real pH value in the ion exchanger phase ($p\bar{H}$) is universal. The fact that the required indicator concentration is by several orders of magnitude lower than the concentration of the other exchanging ions (and may be neglected in a description of the ion exchange equilibrium state) is an undoubtful advantage of the indicator method.

Quite a new approach may be used for determination of pH. For strong acid or strong basic ion exchangers the selectivity concentration constants of the hydrogen ion or hydroxyl ion with respect to the organic ion ($K_H^{org^+}$ or $K_{OH}^{org^-}$) and the constants of K_H^{Na} or K_{OH}^{Cl} are determined and after that pH is estimated according to equation (1a) or (1b) with the use of experimental sorption results:

$$\bar{C}_H = g_0 C_H / [\bar{V}(K_H^{Na} C_{Na} + K_H^{org^+} C_{org^+} + C_H)] \quad (1a)$$

$$\bar{C}_H = \bar{V} C_H (K_{OH}^{Cl} C_{Cl} + K_{OH}^{org^-} C_{org^-})/g_0 \quad (1b)$$

where C_i, $\bar{C}_i$ are the ion concentrations (mg-equiv/ml) in solution and ion exchangers, respectively, g_0 is the total ion exchange capacity of ion exchangers, mg-equiv/g, and $\bar{V}$ is the specific volume of ion exchangers, ml/g.

The method of measuring the hydrogen ion concentration in the phase of weak buffer ion exchangers is based on the use of the concentration form of the Donnan equation. It implies determination of the Donnan exclusion coefficient of the nonexchangeable electrolyte (K_D) expressed as $\bar{C}_{Cl}/C_{Cl}$ for the cation exchanger and as $\bar{C}_{Na}/C_{Na}$ for the anion exchanger as a function of the pH of solution, when the mineral salt concentration is known and the subsequent use of this pH value for an estimation of the real pH (Fig.6) (Refs 24,25):

$$p\bar{H} = -\log(C_H/K_D) \quad (2)$$

We have discussed the characteristic features of the ion exchange of organic ions with the ion exchanger counterions, i.e. the characteristic features of the true ion exchange. However, a great number of natural substances and their synthetic analogues belong to the class of ampholytes and contain zwitterions. Sorption of the ampholytes by ion exchangers involves several processes, i.e. true ion exchange of the ampholyte ions by the ion exchanger counterions, distribution of the zwitterions in water and the sorbent and Donnan exclusion of the ampholyte coion from the ion exchanger.

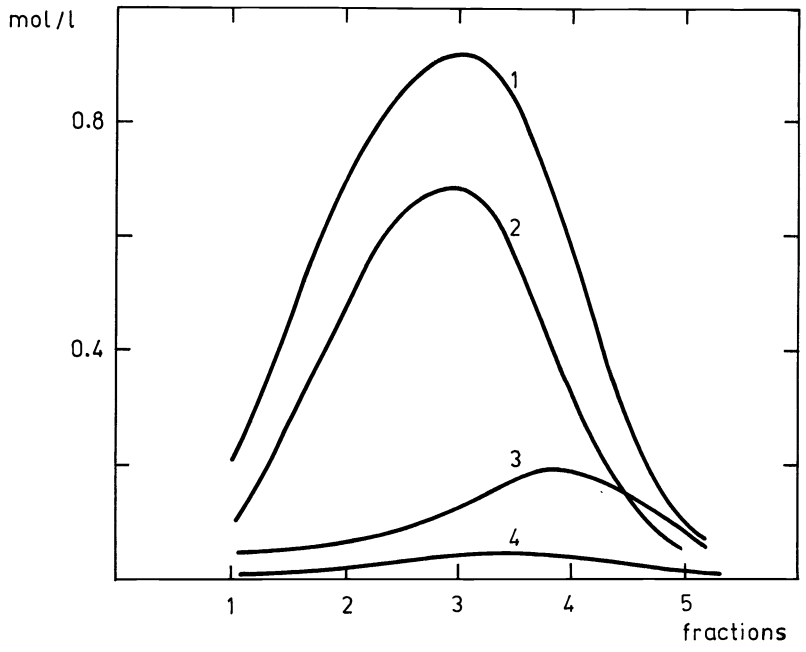


Fig.5. Gentamycin desorption by 0.5 M solution of sulfuric acid. (1,4) Desorption of gentamycin and magnesium after washing of the cation exchanger, (2,3) desorption of gentamycin and magnesium without washing of the cation exchanger.

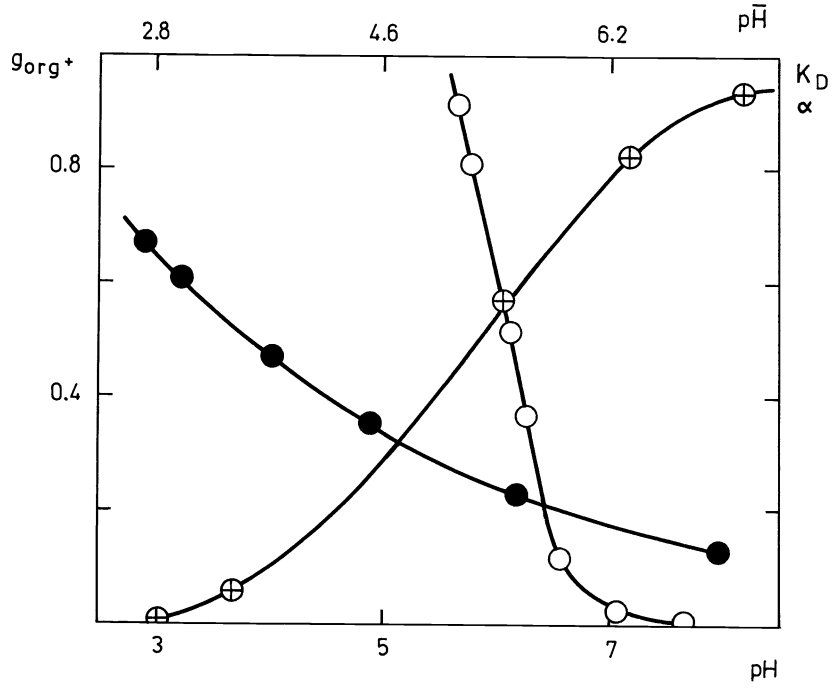
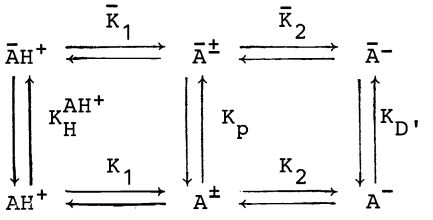


Fig. 6. Ion exchange procedure for determination of the protein isoelectric point (20°C, 0.1 M NaCl, KB-2x0.5, Na-H-form). (1) Dependence of the protein sorption (g_{org}, mg-equiv/g) on pH of the solution and ion exchanger (pH̄), (2) dependence of K_D of NaCl on pH, (3) changes in the ionization level (α) of the carboxylic cation exchanger at pH 3.0-8.0. Arbitrary units.

The theory of the ampholyte sorption developed by us and the method of the equilibrium estimation in such systems allow to determine the equilibrium constants for every ampholyte form and the ionization constants of the ampholytes in the ion exchanger. A quantitative description of the equilibrium state in such systems provides not only the choice of the most rational method for the ampholyte isolation from the fermentation broth, but also a solution of a more complicated problem, i.e. determination of the conditions for chromatographic separation of the mixtures of ampholytes possessing close properties.

Distribution of monoaminomonocarboxylic acid between the strong acid cation exchanger and solution (the most simple case) may be schematically presented as follows:



Cation exchanger

Solution

where A^+, $A^{\pm}$, A^-, $\bar{A}H^+$, $\bar{A}^{\pm}$, $\bar{A}^-$ are cation, zwitterion, ampholyte anion in solution and ion exchanger respectively,

K_i, $\bar{K}_i$ are the ampholyte ionization constants in solution and ion exchanger, respectively (concentrations are given in mol/l),

$K_H^{AH^+}$ is the concentration constant of ion exchange of ampholyte cation - hydrogen ion,

K_p is the distribution coefficient of zwitterion,

K_D is the Donnan distribution coefficient of ampholyte as non-exchangeable electrolyte.

Distribution of separate ampholyte electrochemical forms between the phases may be described by equations (3a-c) for a system containing the cation exchanger and by equations (3b) and (3'a,b) for the anion exchange system:

$$K_H^{AH^+} = g_{AH^+} f(C_H)/g_H C_{am} \qquad (3a)$$

$$K_{Cl}^{A^-} = g_{A^-} C_{Cl} C_H f(C_H) g_{Cl} C_{am} K_1 K_2 \qquad (3'a)$$

$$K_p = g_{A^{\pm}} f(C_H)/\bar{V}\, C_{am} K_1 \qquad (3b)$$

$$K_{D'} = g_{A^-} C_H f(C_H)/\bar{V}\, C_{am} K_1 K_2 \qquad (3c)$$

$$K_{D''} = g_{AH^+} f(C_H)/\bar{V}\, C_{am} C_H \qquad (3'c)$$

where

$$f(C_H) = (C_H^2 + K_1 C_H + K_1 K_2)/C_H$$

$K_{Cl}^{A^-}$ is the concentration constant of ion exchange of ampholyte anion-chloride ion,

C_{am} is the ampholyte analytical concentration in solution, mg-equiv/ml,

g_{am} is the total amount of ampholyte in ion exchanger, mg-equiv/ml,

C_i, $\bar{C}_i$ are the ion concentrations in solution and ion exchanger, mg-equiv/ml, respectively

g_i is the amount of ions sorbed in the ion exchanger phase, mg-equiv/g.

The electrochemical state of the ampholytes in the solution and ion exchanger is described by the respective ionization and balance equations:

$$K_1 = C_{A^\pm}\, a_H / C_{AH^+} \tag{4a}$$

$$K_2 = C_A - a_H / C_{AH^+} \tag{4b}$$

$$\bar{K}_1 = g_{A^\pm}\, g_H / \bar{V}\, g_{AH^+} \tag{4'a}$$

$$\bar{K}_2 = g_A - g_H / \bar{V}\, g_{A^\pm} \tag{4'b}$$

$$g_{am} = g_{AH^+} + g_{A^\pm} + g_{A^-} \tag{5}$$

Combined use of the above equations describing the inter- and intraphase distributions of ampholytes provides relation which indicates that the electrochemical state of ampholytes in solution and ion exchangers is connected with preferable sorption of one of the electrochemical forms of the amphoteric electrolyte:

$$\bar{K}_1 / K_1 = K_p / K_H^{AH^+} \tag{6a}$$

$$\bar{K}_2 / K_2 = K_{OH}^- / K_p \tag{6b}$$

It is evident from the above equations (6) that any three out of the four constants are sufficient for a quantitative description of the system. The succession in which the constants are measured may be arbitrary and depends on the properties of the sorbent and sorbate. The usual procedure is based on measurement of one of the constants of the ampholyte interphase distribution in the presence of one of the electrochemical forms in the ion exchanger phase followed by its use for calculation of the ampholyte ionization constant in the ion exchanger and of the other sorption constant when two ampholyte forms are present in the ion exchanger (Refs 27,28).

In this way, the equilibrium constants were estimated in such a way for systems including various amino acids, peptides, antibiotic ampholytes and their semiproducts, which are also ampholytes and strong acid cation exchangers of different nature, i.e. resins and sephadexes (Refs 28-30). When weak ion exchangers are used, ionization of the sorbent should be considered.

Estimation of the electrochemical and sorption constants allows to choose conditions for the separation of ampholyte mixtures. To solve this problem it is necessary to determine changes in the value of the total amount of the ampholyte sorbed as dependent on pH of the solution. Mineral salts are always present in real systems containing ampholytes which are to be separated. For this reason, the most complicated case is discussed, i.e. sorption of an ampholyte by ion exchangers in mixed forms under conditions involving three processes: ion exchange, distribution of the zwitterions and exclusion of the ampholyte as a nonexchangeable electrolyte. The equation for calculation of the ampholyte cation amount sorbed in a ternary system (H^+, Na^+, AH^+) is

$$g_{AH^+} = K_H^{AH^+}\, A\, C_{am}\, \bar{V} \tag{7}$$

where

$$A = g_0 C_H / [\bar{V} f(C_H) (K_H^{Na} C_{Na} + C_H) + K_H^{AH^+} C_{am} C_H]$$

Equation (7) is established by inclusion of the values

$$g_H = g_0 - (g_{AH^+} + g_{Na}) \text{ and } g_{Na} = K_H^{Na} C_{Na} g_{AH^+} / K_H^{AH^+} C_{AH^+}$$

into equation (3a).

For the anion exchange system the following equation is used:

$$g_{A^-} = K_{Cl}^{A^-} A' C_{am} \bar{V} \qquad (7')$$

where

$$A' = \alpha g_0 K_1 K_2 C_H / [C_{Cl} + K_{Cl}^{A^-} C_{am} K_2 f(C_H)]$$

and α is the ionization degree of the anion exchanger.

For calculation of the amount of the ampholyte zwitterion sorbed the rearranged equation (3b) may be used:

$$g_{A^\pm} = K_p K_1 C_{am} \bar{V} / f(C_H)$$

The amount of the sorbed nonexchangeable electrolyte may be calculated according to equation (8) for the cation exchange system and equation (8') for the anion exchange system:

$$g_{A^-} = K_2 K_p K_1 C_{am} \bar{V} D \qquad (8)$$

$$g_{AH^+} = K_p C_{am} \bar{V} D' \qquad (8')$$

where

$$D = K_H^{Na} C_{Na} \bar{V} / f(C_H) g_0 C_H \quad \text{and} \quad D' = C_H / f(C_H) K_2$$

Finally, the equation for calculation of the total amount of the ampholyte sorbed may be presented as follows:

$$g_{am} = C_{am} \bar{V} [K_H^{AH^+} A + K_p K_1 (B + \bar{K}_2 D)] \quad \text{Cation exchange system} \qquad (9)$$

$$g_{am} = C_{am} \bar{V} [K_{Cl}^{A^-} A' + K_p (K_1 B + D')] \quad \text{Anion exchange system} \qquad (9)$$

where

$$B = 1 / f(C_H)$$

The equilibrium constants of ampholytes in the ion exchanger phase for cefalexin (cel) and its semiproducts, i.e. D-phenylglycine (D-PG) and 7-aminodesacetoxycephalosporanic acid (7-ADCA) is presented in Fig.7. The constants of these substances and their penicillin analogues required for the estimation are presented in Table 1.

The ranges of pH and conditions preferable for ion exchange processes, distribution of ampholyte zwitterions, Donnan exclusion of ampholytes as nonexchangeable electrolytes are evident from the curves presented in Fig.7. The sorption curves are of a significant practical value, since they provide choosing of the optimal conditions for ampholyte sorption and desorption. The optimal pH value of the sorption process depends on K_1 and the mineral ion concentration in the solution.

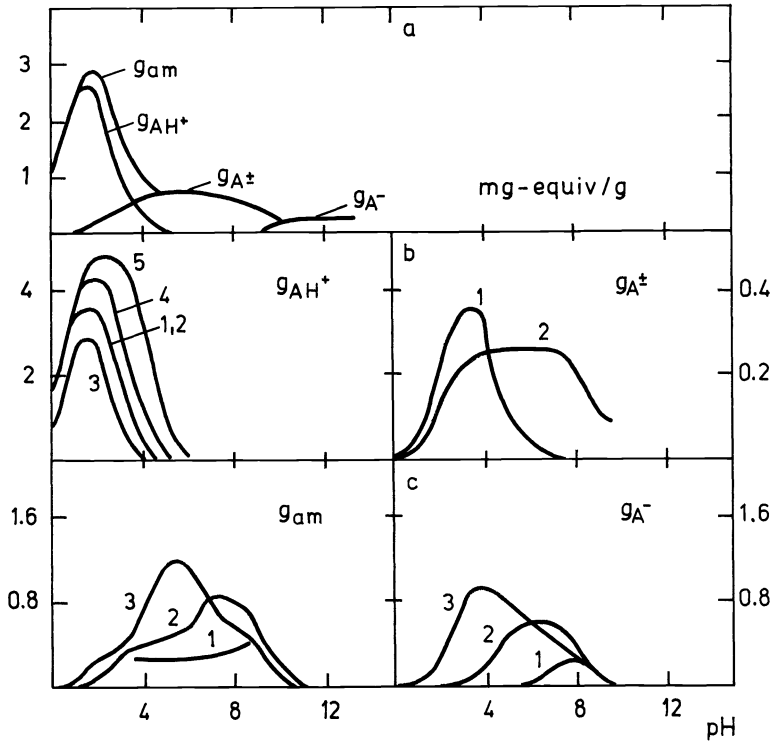


Fig.7. Dependence of the amount of the ampholyte and its separate electrochemical forms in the ion exchanger on pH and concentration of the mineral ion in the solution (20°C); g_i in mg-equiv/g.
(a) D-PG, C=0.05 M, C_{NaCl} = 0.1 M, KU-2-8;
(b) KU-2-8, 1,2-D-PG and 7-ADCA, C = 0.1 M, C_{NaCl} = 0.1 M;
3,4,5-D-PG, C = 0.05 M, C_{NaCl} = 0.1 M, 0.01 M, 0.001 M;
(c) Amberlite IRA-938, 1,2,3-D-PG, cel, 7-ADCA, C = 0.1 M;
C_{NaCl} = 0.1 M.

TABLE 1. Electrochemical and sorption constants of ampholytes

Exchanger		D-PG	7-ADCA	6-APA(28)	Ampicillin	Cefalexin
pK_1		1.96	2.25	2.67	2.38	2.85
pK_2		9.02	4.85	4.71	7.07	7.15
$p\bar{K}_1$	KU-2-4	2.4	-	3.0	2.7	-
$p\bar{K}_1$	KU-2-8	2.6	2.7	-	-	-
$p\bar{K}_2$	IRA-938	9.1	4.8	-	-	7.0
$K_H^{AH^+}$	KU-2-4	3.4	-	2.2	6.3	-
K_p	KU-2-4	1.2	-	0.9	2.9	-
$K_H^{AH^+}$	KU-2-8	4.6	4.0	-	-	-
K_p	KU-2-8	1.0	1.4	-	-	4.6
$K_{Cl}^{A^-}$	IRA-938	0.7	1.0	-	-	1.4
K_p	IRA-938	0.9	1.0	-	-	1.1

With respect to the system containing anion exchangers (Fig.7) the ranges of the ampholyte maximum sorption are connected with the anion exchange region of the curve.

The anion sorption curve also has a maximum controlled by the value of the second constant of the ampholyte ionization when the concentration value of the mineral salts is known. Significant variation of conditions for the sorption of the studied ampholytes is connected with differences in the values of their K_2.

The sorption curves plotted with the use of the electrochemical and sorption constants provide not only choice of the optimal conditions for the isolation of individual ampholytes, but also determination of ways for the chromatographic separation of the ampholyte mixtures.

The affinity levels of sorbates and sorbents are characterized by the value of the effective distribution coefficient equal to the ratio of the total concentration of the substance in the ion exchanger to its concentration in solution (Ref.31):

$$K_p^{eff} = g_{am}/\bar{V}\, C_{am} \qquad (10)$$

When the column parameters are known, K_p^{eff} may be used for predicting the possibility of a chromatographic separation of the substances. By the substitution of g_{am} from equation (9) for cation exchangers and from equation (9') for anion exchangers into equation (10), it is possible to calculate the ratios providing estimation of the effective coefficient of the ampholyte distribution at any pH value and mineral salt concentration by using the sorption and electrochemical constants of the ampholyte determined earlier in the model experiments.

In the system containing a cation exchanger,

$$K_p^{eff} = K_H^{AH^+} A + K_p K_1 (B + \bar{K}_2 D) \qquad (11)$$

and in the system containing an anion exchanger,

$$K_p^{eff} = K_{Cl}^{A^-} A' + K_p (K_1 B + D') \qquad (11')$$

To estimate the possible choice of conditions for the separation of the ampholyte mixture, curves of the $1/K_p^{eff}$ dependence on pH are plotted and the most favourable conditions for the separation of ampholytes are calculated (Refs 32,33).

This method was also used by us for an estimation of the possibility for separation of a mixture of peptides with close ionization constants. Satisfactory coincidence of the calculated data with the experimental findings was observed (Ref.33).

Therefore, a quantitative description of such systems may be used as a basis for the development of procedures for the industrial isolation of amino acids, such as L-lysine, L-triptophane and others, polybasic antibiotics, such as gentamycin and vancomycin, and for complete purification of ampholyte lactam antibiotics, such as cefalexin, ampicillin and others from their semiproducts.

REFERENCES

1. L.F. Yakhontova, E.M. Savitskaya and B.P. Bruns, Zh.Fiz.Khim. 33, 15-18 (1959). (In Russian).
2. G.Ja. Gerasimov, L.F. Yakhontova and B.P. Bruns, Vysokomol.Soed. 2, 864-870 (1960). (In Russian).
3. E.M. Savitskaya, L.F. Yakhontova and B.P. Bruns, Vysokomol.Soed. 2, 1416-1420 (1960). (In Russian).
4. Lou-Dzhi-Syan, E.M. Savitskaya and B.P. Bruns, Dokl.Akad.Nauk SSSR 136, 151-154 (1961). (In Russian).

5. G.S. Libinson, E.M. Savitskaya and B.P.Bruns, Zh.Fiz.Khim. 37, 641-643, (1963). (In Russian).
6. E.S. Vaisberg, L.F. Yakhontova and B.P. Bruns, Zh.Fiz.Khim. 41, 892-895, (1967). (In Russian).
7. E.S. Vaisberg, L.F. Yakhontova and B.P. Bruns, Zh.Fiz.Khim. 41, 2962-2964 (1967). (In Russian).
8. E.S. Vaisberg, L.F. Yakhontova and B.P. Bruns, Zh.Fiz.Khim. 40, 2953-2956 (1966). (In Russian).
9. E.S. Lyustgarten, V.N. Li and A.B. Pashkov, Plastmassy 5, 7-15 (1964). (In Russian).
10. I. Saidl, Ya. Malinski and K. Dushek, Plastmassy 12, 7-15 (1963).
11. L.F. Yakhontova, N.A. Perevozskaya, S.N. Kobzieva and others, Khim.Farm.Zh. 3, 111-115 (1976). (In Russian).
12. V.S. Guzik, A.A. Sandar, M.P. Kovaleva and V.P. Slugin, Ion Exchange and Chromatography, Part 2, Vp 29-30, Voronezh State University Publishing House, Voronezh (1971). (In Russian).
13. N.A. Perevozskaya, L.F. Yakhontova, B.P. Bruns and N.L. Isaeva, Ion Exchange and Chromatography, Part 2, p.14, Voronezh State University Publishing House, Voronezh (1971). (In Russian).
14. E.M. Savitskaya, L.F. Yakhontova and P.S. Nys, Ion Exchange, p. 229-248, Nauka, Moscow (1981). (In Russian).
15. L.F. Yakhontova, E.S. Vaisberg and S.N. Kobzieva, Khim.Farm.Zh. 11, 32-35 (1975). (In Russian).
16. L.F. Yakhontova, V.M. Fishman, N.A. Perevozskaya and S.N. Kobzieva, Khim.Farm.Zh. 6, 84-87 (1976). (In Russian).
17. L.F. Yakhontova, V.M. Fishman and N.A. Perevozskaya, Khim.Farm.Zh. 3, 111-115 (1976). (In Russian).
18. L.F. Yakhontova, B.P. Bruns, S.N. Kobzieva and N.A. Perevozskaya, Antibiotiki 5, 411-415 (1970). (In Russian).
19. E.M. Savitskaya, N.N. Shellenberg and D.I. Shvedov, Med.Prom. 4, 13-17 (1960). (In Russian).
20. K.I. Surkova, E.M. Savitskaya and B.P. Bruns, Ion Exchange Technology, p. 136-140, Nauka, Moscow (1965). (In Russian).
21. P.S. Nys and E.M. Savitskaya, Physical Chemistry of Solutions, p. 254-259, Nauka, Moscow (1972). (In Russian).
22. P.S. Nys and E.M. Savitskaya, Dokl.Akad.Nauk SSSR 176, 873-875 (1967). (In Russian).
23. P.S. Nys, E.M. Savitskaya and T.S. Kolygina, Khim.Farm.Zh. 9, 58-62 (1971). (In Russian).
24. E.M. Savitskaya, P.S. Nys and N.N. Shellenberg, Antibiotiki 2, 121-125 (1977). (In Russian).
25. P.S. Nys and E.M. Savitskaya, Zh.Fiz.Khim. 43, 1536-1540 (1969). (In Russian).
26. E.M. Savitskaya, P.S. Nys and B.P. Bruns, Dokl.Akad.Nauk SSSR 164, 378-381 (1965). (In Russian).
27. E.M. Savitskaya and P.S. Nys, Kunstharz-Ionen-Austauscher, p.403-414, Akademie-Verlag, Berlin (1970).
28. E.M. Savitskaya, P.S. Nys and M.S. Bulycheva, Khim.Farm.Zh. 7, 32-38 (1969). (In Russian).
29. M.S. Bulycheva, P.S. Nys and E.M. Savitskaya, Zh.Fiz.Khim. 44, 1124-1126 (1970). (In Russian).
30. R.M. Petyushenko, E.M. Savitskaya and P.S. Nys, Zh.Fiz.Khim. 49, 422-427 (1975). (In Russian).
31. F. Wilson, J.Amer.Chem.Soc. 62, 1583 (1940).
32. M.S. Bulycheva, Dissertation. Moscow State University, 1972. (In Russian).
33. P.S. Nys, E.M. Savitskaya and R.M. Petyushenko, Zh.Fiz.Khim. 49, 2330-2334 (1975). (In Russian).