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CHROMATOGRAPHY
OF POLIMERIZATION-
CAPABLE SURFACE-ACTIVE SUBSTANCES

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INTRODUCTION

The regularities of classic chromatography are valid also in high-performance liquid chromatography (HPLC) - method, where the selection of the conditions for experiment is specific and depend on the sorbent type, nature of the mobile phase, its composition and other parameters.

The studies of newly synthesized organic compounds by liquid chromatography are mainly related to the search for regularities among their sorption, structure, as well as physicochemical characteristics. Obtained research findings allow to solve tasks targeting the search of functional dependencies of the elution parameters, thus enabling to control the process of separation, to optimize it and to use it successfully for the identification of the individual components.

The **aim** of the present study was to investigate the possibility of the analysis of maleic acid derivatives by means of reversed, normal-phase and ion exchange HPLC methods, as well as to evaluate the contribution of hydrophobic and hydrophilic nature of sorbates in separation parameter values, by using Reker's additivities of hydrophobicity constants, hydrophobic-lipophilic balance, Lanin's quasichemical equilibration model, as well as Levin's model of a unified adsorption centre.

The **tasks** of the study comprised:

- measurements of the retention parameters, by mean of various HPLC methods,
- the development of appropriate conditions of chromatographic analysis for the identification of oligomaleate mixture,
- the application of the results obtained in the evaluation of sorption regularities and focused selection of the analysis conditions.

The **topicality** of the research was determined by the necessity to develop methods of the analysis of double bond containing nonionic and cation maleic acid derivatives, as well as by the necessity to control the results of synthesis and structure of the products.

The novelty of the present research is related to the application of HPLC technology in defining the regularities of the retention of newly synthesized maleic acid derivatives, by using silica, octadecyl silica and stationary phases containing carboxygroups with appropriate mobile phase.

The **practical significance** of the research is related to the application of maleic acid derivatives in separation of surface-active substances – during their polymerization process stable emulsions with low surface tension between disperse phase and dispersion

environment are derived. Emulsions, owing such quality, are capable to generate latexes that are sustainable against the environmental impact and as a result to improve the quality of products made of polymers.

During analysis of these compounds, chromatographic researchers specify the regularity of sorption in the multicomponent systems of systematization that is directed towards its practical application by solving ecological and technical issues. This process is especially important for the characterization of the sorption of newly synthesized compounds and for the validation of their identity, since it ensures the conversion from empirical approach in the selection of conditions to the targeted application of regularities, thus constituting background for the non-standard qualitative analysis.

The content of the thesis is composed of introduction, review, experimental, results and discussion, list of literature and appendixes.

In the review of literature methods of determination of surface-active substances by means of normal and reversed phase, as well as ion-exchanges under HPLC conditions are outlined. The author describes studies of regularities between the sorption of the studied compound, its structure and physico chemical properties.

The experimental part describes the literature used as reference in this study, methodology of chromatographic measurements, equations applied in the calculations and model equations.

In the part of results and discussion the author evaluates the efficiency of several sorption systems for qualitative analysis of maleic acid derivatives, as well as provides an evaluation of the characteristic values of sorption and the effectiveness of the studied systems with respect to the investigated compounds.

RESULTS AND DISCUSSION

1. Chromatography of maleic acid diester derivatives

Alkyl(polytrihydroxypropyl)maleate mixture is derived as a products obtained in the heating reaction of maleic acid hemiester and glycidol in inert atmosphere, and without use of any solvents. The derived composition consists of compounds with hydrophobic substituent and differing hydrophilic part – 2,3-dihydroxypropyl group or poly(2-hydroxypropyloxy) (**3**) as well 1,3-dihydroxyprop-2-yl group or poly(1-hydroxymethylethyloxy) groups (**4**) (Fig.1).

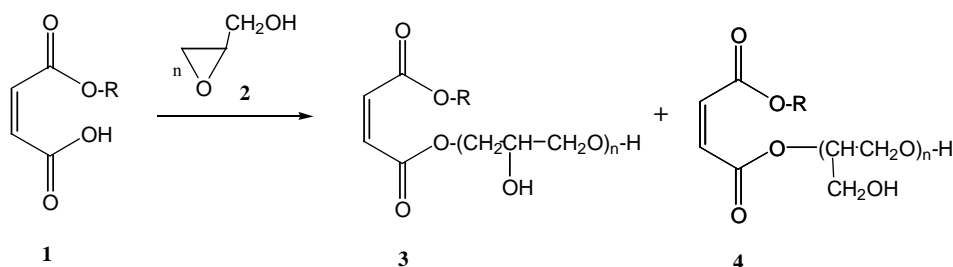


Fig. 1. Formation reaction and structures of discussed non-ionic surfactants - alkyl(polytrihydroxypropyl)maleates **3**, **4**: $R = C_{12}H_{25}$; $R = C_{16}H_{33}$; $R = C_{18}H_{37}$; $n = 1-4$.

Performed HPLC analysis under reversed phase (RP) conditions, revealed a linear correlation among the retention factors $\lg k$ of alkyl(polytrihydroxypropyl)maleates and their number of hydroxypropyloxy groups, thus confirming the belonging of mixture components to a certain tail of oligomers. It is found that, by using methanol as a mobile phase, the tail of oligomers separates from the column according to the sequence of the decrease in the number of added hydroxypropylgroups, starting with $n = 4$ (the largest number of –OH groups) and ending with $n = 1$ (Fig. 2).

In HPLC RP system the resolution R_S of alkyl(polytrihydroxypropyl)maleate homologs is approximately 1.0. Complete retention ($R_S > 1.5$) is reached by means of normal-phase sorbent, using gradient elution: 2-propanol/n-hexan/diethyl ether from 5:35:60 up to 40:0:60 (Fig. 3).

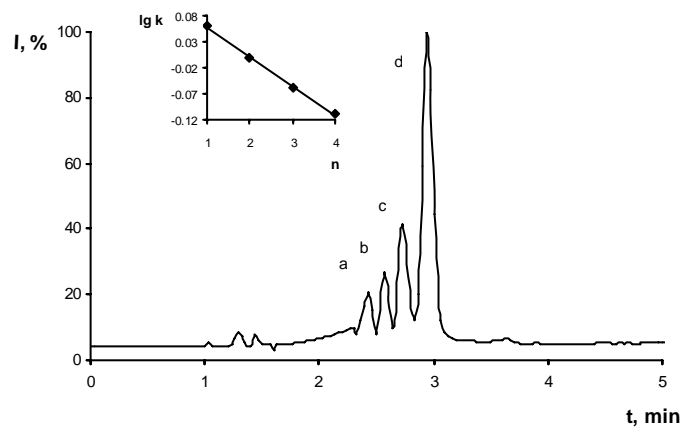


Fig. 2. Reverse-phase separation of dodecyl(polytrihydroxypropyl) maleates **3+4** on a Zorbax SB-18 column and methanol as the mobile phase. The sequence of peaks corresponds to the number of 2-hydroxypropuloxy units: $n=4$ (a), $n=3$ (b), $n=2$ (c), $n=1$ (d).

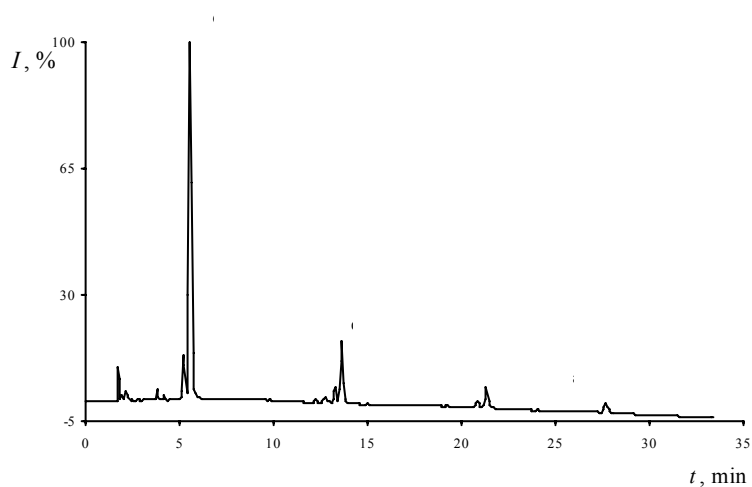


Fig. 3. Normal-phase separation of dodecyl(polytrihydroxypropyl) maleates **3+4** on a Zorbax RX-SIL column, using gradient elution (2-propanol/*n*-hexane/diethyl ether from 5/35/60 \rightarrow 40/0/60) during 40 min. The sequence of peaks corresponds to the number of 2-hydroxypropuloxy units: $n = 1$ (d), $n = 2$ (c), $n = 3$ (b), $n = 4$ (a).

Under the selected conditions of absorption, compounds were eluted according to the sequence of the increase in their polarity with maximum retention time tetra-values ($n = 4$), however, oligomers having a number of hydroxypropyloxi-groups higher than $n = 4$ were not found. The content of each homolog in composition is calculated according to the normalized height of peaks. Approximate content of investigated alkyl-(polytrihydroxypropyl)maleate monomers was 68%, that of dimer 12%, trimer 5% and tetramer 1% .

In order to obtain an additional confirmation of the structure of retained compounds, individual homologs were isolated on preparative scale from the composition under HPLC condition, and they were further converted into the corresponding trimethylsilyl (TMS) derivatives. In gas chromatography peaks were obtained using a high-performance capillary column *DB-5MS* (Fig. 4), and their corresponding mass spectra confirmed the composition of expected oligomaleates. The observed signals M-15 and M-103 in the mass spectra of trimethyl-silylated surfactants clearly indicate the molecular ion masses of maleic acid diesters of TMS derivatives (Fig. 5).

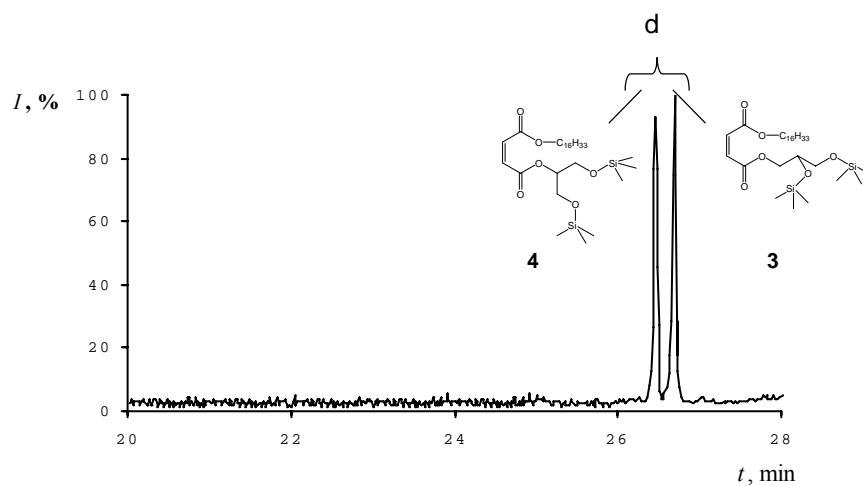


Fig. 4. GC chromatogram of TMS derivatives of hexadecyl(1,3-dihydroxyprop-2-yl) (4) and hexadecyl(2,3-hydroxypropyl)maleate (3) obtained after preparative separation of the peak (d) (Fig. 3) with further trimethylsilylation.

One possibility of their formation might be the charge-site driven C–O⁺ cleavage found closer to the ester group in the investigated compound (Fig. 5).

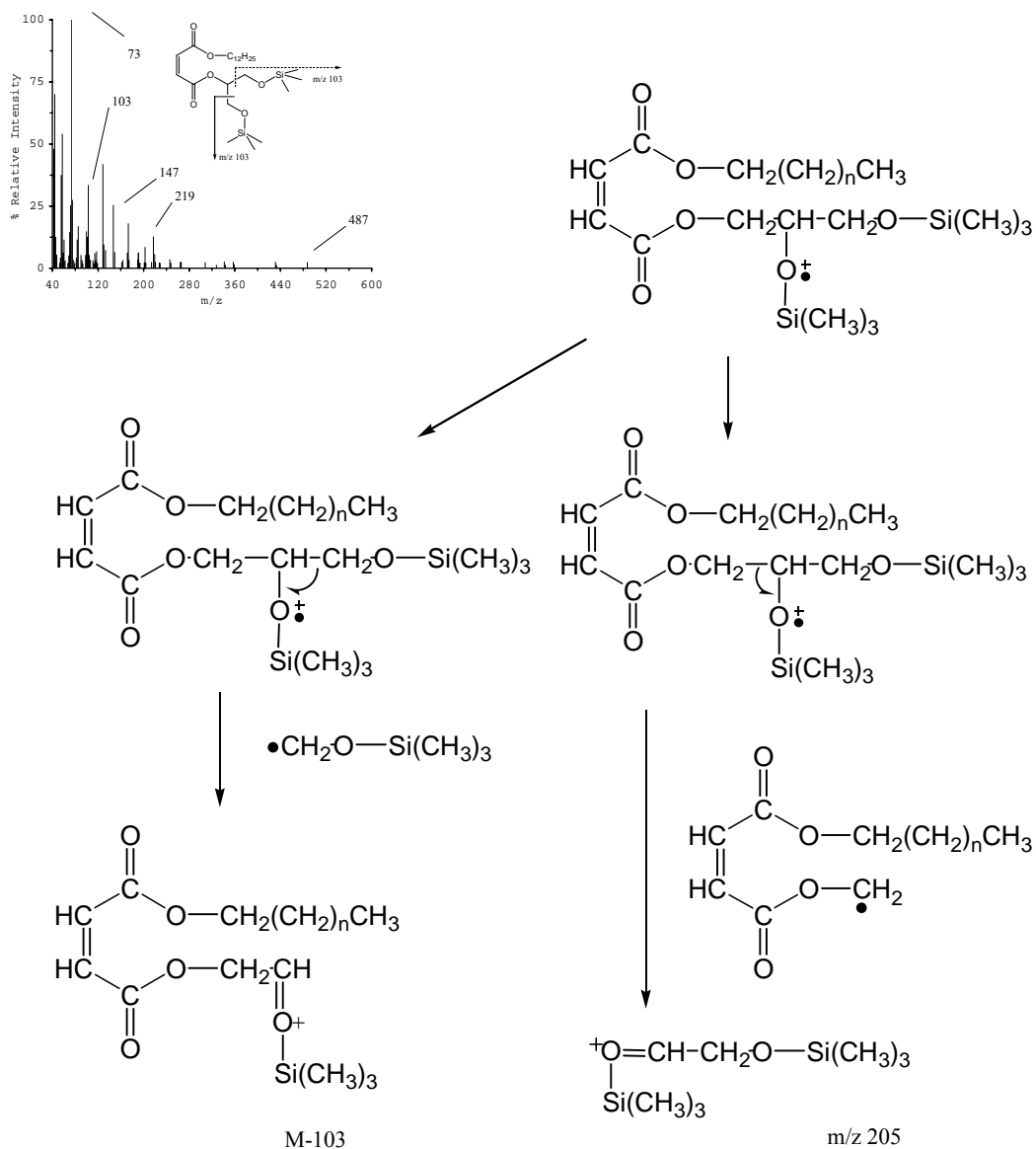


Fig. 5. Electron impact (70eV) mass spectra of TMS derivatives of maleic acid diesters and possible formation of ions m/z M-103 and m/z 205.

2. The analysis of maleic acid diamide derivatives and studies of sorption in reversed-phase conditions

The parameters of maleic diamide (Fig. 6) sorption are calculated, by using several sorbents hydrophobized with octadecyl groups and mobile phases containing methanol, acetonitrile and tetrahydrofuran.

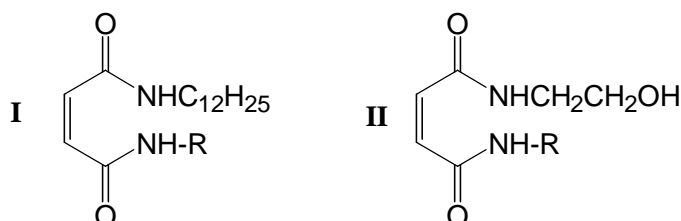


Fig. 6. Investigated derivatives of maleic acid diamides. **I**: R –CH₂CH₂OH (**5**); –CH₂CH₂OCH₂CH₂OH (**6**); –CH₂CH₂OCH₃ (**7**); –C(CH₂OH)₃ (**8**); –CH₂C₆H₅ (**9**); **II**: R –C₆H₄Cl (**10**); –CH(CH₃)CH₂CH₃ (**11**).

It was found that, in order to reach an optimal ($1 < k < 10$) chromatographic retention of these compounds, mobile phases with high content of organic modifier should be used. It was observed that optimal k values can be reached by means of 70–80% methanol solutions in water in mobile phase (Fig. 7). k values considerably increase along with the decrease by 40 – 50 % of methanol content in eluent, thus making the analysis of maleic acid diamides difficult.

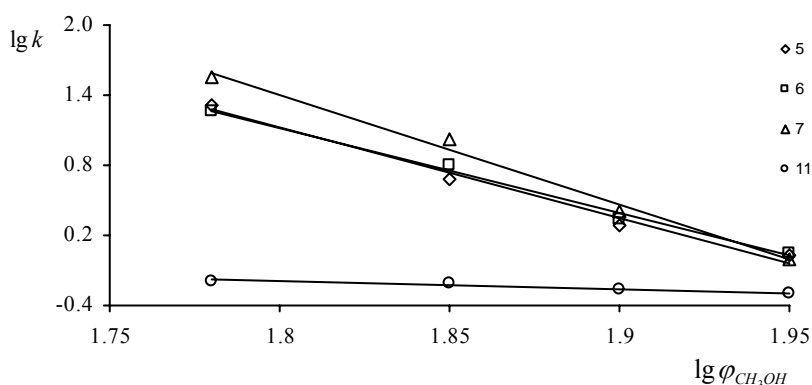


Fig. 7. Dependence of the $\lg k$ on $\lg \varphi_{\text{CH}_3\text{OH}}$ for the derivatives of diamides of maleic acid investigated. Maleic dodecyl diamides: **5** – 2-hydroxyethyl, **6** – 2-(2'-hydroxyethoxy)ethyl, **7** – 2-methoxyethyl; maleic 2-hydroxyethyl diamide: **11** – sec-butyl.

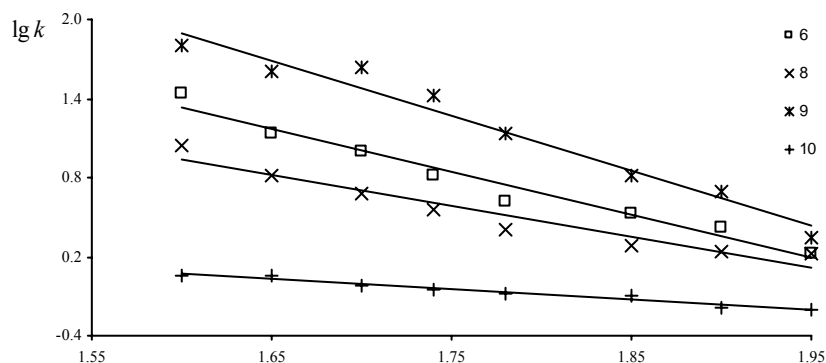


Fig. 8. Dependence of $\lg k$ on the $\lg \varphi_{\text{CH}_3\text{CN}}$ for derivatives of diamides of maleic acid investigated. Maleic dodecyl diamides: **6** – 2-(2'-hydroxyethoxy)ethyl, **8** – tris(hydroxymethyl)methyl, **9** – benzyl; maleic 2-hydroxyethyl diamide: **10** – *p*-chlorophenyl.

The concentration range of mobile phase in the analysis of the above mentioned substances at 60 – 70% CH₃OH content corresponds to approximately 50 – 60% CH₃CN water solutions (Fig. 8). Diamides have the lowest capacity factors k of all used eluent compositions, containing the number of polar groups that exceeds that of less polar (hydrophobic) substituents.

Diamide capacity factor k values of the studied maleic diamides are differing, when using different types of reverse phase sorbents. Higher k values are obtained with sorbent *Zorbax SB C₁₈* than *Nucleosil 100-5 C₁₈* and *Nova-Pak C₁₈*, using CH₃CN and CH₃OH as a modifier. The k values of substances are significantly lowered, when methanol is used as organic modifier. This could be related to the differing selectivity of the different sorbents, depending on the substances to be separated, that is constituted by the content of the organic part bound with silica, as well as by the concentration of free silyanol groups.

The results indicate the changing influence of modifier in column efficiency N_{theor} of the studied mobile phase contents. Higher efficiency values were observed with the maleic diamide, when acetonitrile is used in the content of the mobile phase.

The dependence of capacity factors of maleic diamides on tetrahydrofuran content in the mobile phase shows that this organic additive to eluent is not convenient for analysis of these substances.

The contribution of hydrophobic and hydrophilic nature of sorbates to the retention parameter values k was tested, by using Reker's additivities of hydrophobicity constants, hydrophobic-lipophilic balance and the characteristic criteria of hydrophobicity. However, it was detected that these parameters do not tend to form strongly correlational relationships.

By considering adsorption as quasichemical process of reverse interchange, the retention of compounds in various cases of intermolecular interaction sorbate/mobile phase/stationary phase is described by equation offered by Lanin:

$$\frac{1}{k} = \left(\frac{1}{\Phi K_S} \right) \cdot \left(1 + \frac{K_{SM} M_m}{L} \right) \cdot \left[1 + (K_M - 1) M_m - \frac{K_{SM} S_m M_m}{L^2} - \frac{K_{MM} M_m (1 - L^2)}{L^2} \right], \quad (1)$$

where K_S – sorbate molecule sorption quasi-chemical equilibrium constant; K_M – equilibrium constant of modifier sorption; K_{SM} – equilibrium constant of sorbate and modifier association in solution; K_{MM} – equilibrium constant of self-association of modifier; S_m , L , M_m – mole fraction of sorbate, water and modifier in the mobile phase, correspondingly; Φ – interfacial distribution in column.

Deviations from the linear correlation indicate specific and non-specific commensurability of the molecules of sorbate and mobile phase with the non-specific interaction of the molecules of sorbates with hydrophobic surface of stationary phase. The data of the analysis on the correlation of the retention factors of the studied compounds indicate that non-linear relation between $1/k$ and N can be explained by the fact, that, without sorbates (S) and modifier (M) in adsorption-desorption processes (K_S , K_M) in monomolecular sorption layer, a formation of associates is possible between the molecules of sorbate and modifier (S-M), characterized by K_{SM} values.

The interaction of modifier and water significantly increases the contribution of hydrophobic effect, that is reflected in the expression of sorption constants K_S of sorbate in figures (Table 1). Association K_{MM} of molecules of the modifier does not render apparent influence in the investigated eluent systems.

The constant of solvation of molecules K_{SM} for some maleic acid derivatives, in system acetonitrile water is, more, than in system methanol/water, it results in corresponding propagation of retention of derivants in water/methanol eluent at small concentration of the modifier that corresponds to his eluating force.

Table 1

Quasium-chemical equilibrium constants of maleic diamides, achieved using Nucleosil 100-5 C_{18} as stationary phase

Sorbate	K_M	K_{MM}	K_{SM}	K_S	$1/k$	K_M	K_{MM}	K_{SM}	K_S	$1/k$
	Acetonitrile/water					Methanol/water				
5	51	0.95	0090	260	0.36	420	5.0	290	$8.9 \cdot 10^5$	0.52
6	73	0.99	0.34	490	0.38	400	3.9	260	$8.9 \cdot 10^5$	0.46
7	3.2	0.01	37	850	0.37	800	0.00	100	$8.9 \cdot 10^5$	0.40
8	75	2.2	0.17	260	0.58	52	0.57	0.80	$1.0 \cdot 10^3$	0.24
9	10	0.16	5.6	800	0.11	17	0.37	15	$5.0 \cdot 10^3$	0.22
10	0.78	0.0	0.83	3.8	1.4	120	2.1	0.26	280	1.2
11	0.56	0.0	0.35	2.4	1.5	170	3.9	0.29	240	1.8

It results in more abrupt propagation of dependence $1/k-N$ for modifier - methanol in comparison with acetonitrile. In result, at high concentration of methanol in a mobile phase, interaction of molecules of maleic acid derivatives with a solution amplifies and retention in eluent system methanol - water become less, than in system acetonitrile/water. Thus the account of the interactions resulting in a solution to formation of associates, allows to explain character of a deviation of dependence $1/k-N$ from linearity (Fig. 9).

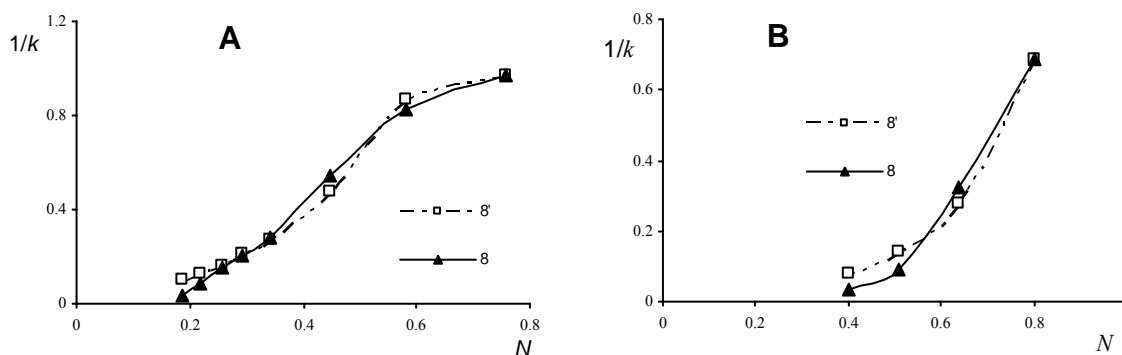


Fig. 9. Dependence of experimental δ and calculated δ' values of reverse retention factors $1/k$ for maleic N -dodecyl, N' -tris(hydroxymethyl)methyl diamides on mole fraction of N modifier in the mobile phase in eluent systems: **A** – acetonitrile/water and **B** – methanol/water.

3. The sorption of quaternary maleic acid derivatives under RP conditions with non-ionic mobile phase

The sorption of several quaternary maleic acid derivatives (Fig. 10) was studied in the system of octadecylsilicagel/methanol/phosphate buffer solution. The retention factors k of the studied compounds indicate that the chromatographic separation of the studied substances is possible with eluents containing part of organic additives that exceeds 70 %. This might be determined by the hydrophobic alkyl groups in the studied derivation of maleic acid.

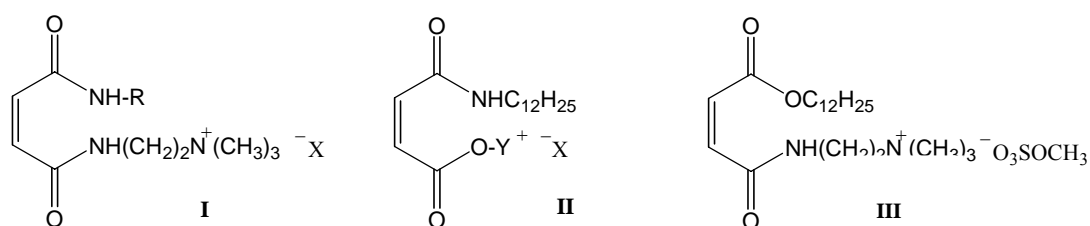


Fig. 10. Investigated derivatives of quaternary maleic acid: **I**: $X = p\text{-CH}_3\text{C}_6\text{H}_4\text{SO}_3$, $R = \text{C}_{12}\text{H}_{25}$ (**12**); $X = \text{CH}_3\text{OSO}_3$, $R = \text{C}_{12}\text{H}_{25}$ (**12a**); $X = p\text{-CH}_3\text{C}_6\text{H}_4\text{SO}_3$, $R = \text{C}_6\text{H}_5$ (**13**); **II**: $X = p\text{-CH}_3\text{C}_6\text{H}_4\text{SO}_3$, $Y = (\text{CH}_2)_2\text{O}(\text{CH}_2)_2\text{N}^+(\text{CH}_3)_3$ (**14**); $X = p\text{-CH}_3\text{C}_6\text{H}_4\text{SO}_3$, $Y = (\text{CH}_2)_2\text{N}^+(\text{CH}_3)_3$ (**15**); **III**: (**16**).

It is observed that in whole studied pH value interval the value k of compounds increases, along with the increase of water content part in the system. Their dependence from the concentrations of methanol is almost linear $r = 0.990 - 0.999$ in whole studied pH interval. The value pH of the mobile phase considerably influences retention factors – their values increase, whereas the eluent changes from acid to alkaline (Fig. 11).

The column efficiency N_{theor} against the studied maleic acid derivatives is 200–1500 theoretic plates, provided the dimensions of the column are $4.6 \times 150 \text{ mm}$, and it depends to a great extent on the structure of the mobile phase – it is significantly influenced by the volume part of methanol and water at pH of phase (Fig. 12).

The measured values of peak asymmetry are high (2.8 – 5.3), thus indicating variety of intermolecular interaction during sorption process. Apart from hydrophobic influences, probably –NH– groups participate in the formation of hydrogen bonds with acid silanol groups of sorbent surface. This contribution increases along with the increase of alkaline content of environment, being especially explicit at pH = 6.2.

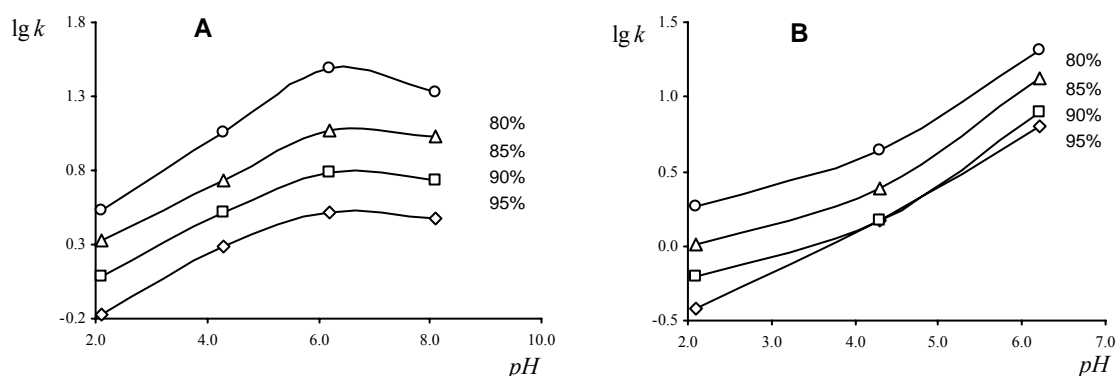


Fig. 11. The dependence of value $\lg k$ of sorbate retention factor on a pH at different concentration of methanol in the mobile phase: **A** – dodecyl-*N*-[2-(*N*',*N*',*N*'-trimethylammonio)ethyl]maleic methylsulfate (**16**), **B** – [2-(*N*,*N*,*N*-trimethylammonio)ethyl]-*N*'-dodecylmaleic 4-methylbenzenesulfonate (**15**).

Furthermore, C=O groups at quaternary maleic acid derivatives and different surface silanols may form differing energy hydrogen bonds, that are capable of providing certain contribution to the variety of interactions and value of peak asymmetry. The interactions N^+ and Si-O⁻ in quaternary maleic acid derivatives can not be excluded either during their increase.

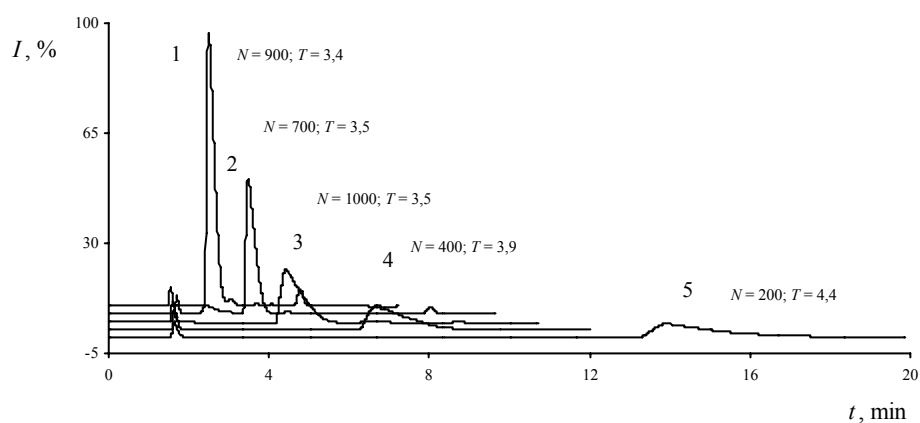


Fig. 12. Chromatograms of the {2-[2'-(*N*,*N*,*N*-trimethylammonio)etoxy]ethyl}-*N*'-dodecyl maleamate 4-metylbenzenesulfonate (**14**) with different content of the methanol in mobile phase: 1 – 95%; 2 – 90%; 3 – 85%; 4 – 80%; 5 – 75%. Column: ZORBAX SB-C18 (4.6×150 mm), mobile phase - $CH_3OH/0.01 M KH_2PO_4/H_3PO_4$ water solutions (pH=2.1).

Levin's model is used for the identification of the nature of the sorption of quaternary maleic acid derivatives under RP conditions, comprising the influence of both mobile phase components. In this model adsorption is viewed as equilibrium among molecules in liquid and on individual adsorption centers on sorbent surface. This concept serves as the basis for the theory of stoichiometric adsorption, where adsorption is regarded as a process of quasicheical association of sorbate to the active surface centers. Granted that the concentration of adsorption centers remains unchanged, along with the change of mobile phase structure, when active components have solved them completely, an equation on single adsorption center model is obtained:

$$\lg k = a_0 + a_1 \lg \varphi_1 + a_2 \lg \varphi_2, \quad (2)$$

where φ_1 and φ_2 – concentration of modifier and water in mobile phase;
 a_0 , a_1 and a_2 – coefficients of equation.

Experimentally calculated retention factor values at different structure φ of the mobile phase (CH₃OH/H₂O) and different pH values of buffer solution are used for the calculation of equation constants.

The equation constant a_2 reflects the contribution of normal-phase mechanism (active component – water). The values of the contribution of non-polar sorbates are close to zero, but, if they contain polar groups, a_2 becomes different from zero and is comparable with a_1 . The calculated values of the constant a_2 of the studied compounds differ from zero relatively little, thus it is the indicative of the poor association of polar groups in the process of sorption (Table 2). The above mentioned allows to assume, that the sorption of compounds is mainly determined by the hydrophobic group included in its composition.

At inclusion in the equation of regression alongside with the contents of the modifier of a mobile phase also drive, sizes of factors of correlation grow at simultaneous increase in the F -factor.

By the data interpolation, it is possible to calculate the values of compound retention factor at any concentration of modifier and to optimize the effectiveness and selectivity of separation by means of correlation (2), using the known planning methods of experiment.

Table 2

Characteristics of Levin's (A) and Soczewinski (B) equations, determined on Zorbax SB-C18 with CH₃OH/H₂O as mobile phase (pH=4.3)

Sorbate	A					B			
	$\lg k = a_0 + a_1 \lg \varphi_1 + a_2 \lg \varphi_2$					$\lg k = a_0 + a_1 \lg \varphi_1$			
	$-a_0$	$-a_1$	$-a_2$	r	F	a_0	$-a_1$	r	F
12	1.3	13	0.94	0.996	664	-0.080	7.7	0.953	80
12a	1.3	13	0.94	0.996	664	-0.080	7.7	0.953	80
14	0.57	9,2	0.35	0.976	114	-0.16	8.3	0.965	109
15	1.1	12	0.75	0.985	126	0.05	6.7	0.956	88
16	0.46	13	0.36	0.997	560	0.040	11	0.993	438

4. The sorption of quaternary maleic acid derivatives under RP ion-pair formation conditions

The dependence of ion-pair retention factors from the concentration of methanol in eluent and pH under HPLC conditions was studied. Sodium hexanesulfonate was used as ion-pair agent.

High composition of organic part in mobile phase is necessary for the separation of compounds. When increasing the volume part of organic additive in eluent, the retention factor values of all studied maleic acid derivatives decrease. The dependence of the retention factors of maleic acid derivatives from the content of methanol in mobile phase forms a linear correlation ($r = 0.990 - 0.999$) between $\lg k$ and $\lg \varphi_{\text{CH}_3\text{OH}}$ (Fig. 13). This correlation allows to assume, that sorption on the modified surface of sorbent is slower when the pH value of methanol is increased from 2.1 to 5.0.

The values of retention factors are higher by approximately 10% when ion-pair agent is added to the mobile phase.

The highest effectiveness against the studied compounds of all investigated systems was reached by the use of ion-pair systems. Mobile phases with 80 % methanol content can be used for optimal retention ($1 < k < 10$) of the studied compounds.

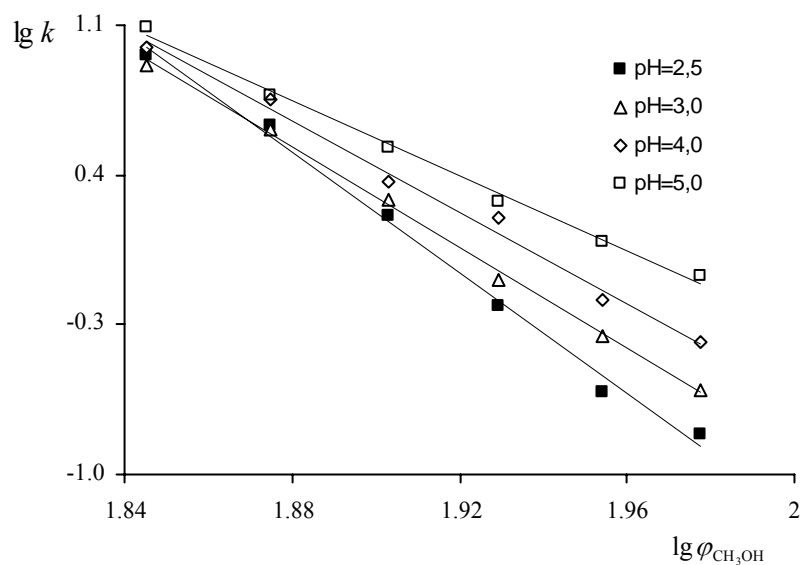


Fig. 13. Dependence of $\lg k$ of dodecyl-*N*-[2-(*N'*,*N'*,*N'*-trimethylammonio)ethyl]maleic methyl sulfonate (**16**) on a $\lg \varphi_{\text{CH}_3\text{OH}}$ at the different pH of mobile phase.

With the aim to describe the change in free energy of chromatographic system in the transition of compound from the system with pH = 2.1 to the system with pH = 5.0 under ion-pair conditions or under conditions without adding ion-pair agent, the values of Gibbs' energy changes $\Delta(\Delta G)$ were calculated according to the equations:

$$-\Delta(\Delta G)_{IP} = RT \ln \frac{k_{pH=5.0}}{k_{pH=2.1}}, \quad (3)$$

where $k_{pH=5.0}$ and $k_{pH=2.1}$ values of the retention factor on a ion-pair reverse-phase systems at pH = 5.0 un pH = 2.1.

The calculated values describe the excess sorption free energy, using mobile phase of different composition (Table 3). The positive changes in the sorption free energy indicate that in used methanol concentration interval *N'*-dodecyl-[2-(*N''*,*N''*,*N''*-trimethylammonio)ethyl]diamide 4-methylbenzenesulfonate (**12**) and dodecyl-*N*-[2-(*N'*, *N'*, *N'*-trimethylammonio)ethyl]maleamate methylsulfate of maleic acid (**16**), as compared to other compounds, consumes less inner energy for the process of sorption.

Table 3

Values of the sorption free energy change $\Delta(\Delta G)$ (kJ/mol) of investigated quaternary maleic acid derivatives in a ion-pair reverse-phase systems, using methanol/10 mM sodium hexanesulfonate solution

Sorbate	$\varphi_{\text{CH}_3\text{OH}}$, vol. %					
	95	90	85	80	75	70
12	3.0	2.5	1.8	1.7	1.3	0.7
16	4.8	3.7	3.1	2.5	2.1	2.0
15	- 2.4	- 1.8	- 1.7	- 1.8	- 2.0	- 1.5
14	- 2.2	- 2.1	- 1.9	- 1.8	- 2.2	- 1.8

In the case of {2-[2'-(*N*, *N*, *N*-trimethylammonio)etoxy]ethyl}-*N*'-dodecylmaleamate 4-methylbenzenesulfonate (**14**) and [2-(*N*,*N*,*N*-trimethylammonio)ethyl]-*N*'-dodecylmaleamate 4-methylbenzenesulfonate of maleic acid amidesters (**15**), the adsorption energy of compounds exceeds the presumable loss of entropy ($|\Delta H_{ads}| > |T\Delta S_{ads}|$) at pH = 5.0 and, when $\Delta H_{ads} < 0$, then $\Delta G_{ads} < 0$). In this case compounds, by means of transition from mobile phase with pH = 5.0 to pH = 2.1, significantly decrease their retention abilities. There are actually no changes in the values of the sorption free energy, when methanol content in mobile phase decreases.

5. Quaternary maleic acid derivatives under HPLC cation-exchange conditions

Evaluating the changes of mobile phase composition, it was observed that acetonitrile tends to decrease significantly the retention times of compounds and tailing peak values, when the admixtures of citrate buffer solution and acetonitrile are used as mobile phase. Retention times of compounds will decrease non-linearly ($r = 0.57 - 0.97$), along with the increase of acetonitrile concentrations. Compounds separate as close, relatively symmetric peaks at 20% CH₃CN. It is observed that, by the increasing of eluent pH, the values of the retention factors increases if the composition of other components remains unchanged. The highest values of the retention factors are founded at pH = 5.5, and this phenomenon being explained by the growing contribution of ion-exchange to the sorption process. Supposedly, most alkaline quaternary maleic acid salts produce higher *k*

values.

Under conditions when ionic carboxyl groups (COO^-) at the matrix of sorbent are in partially ionized form, a low contribution of ion-exchange is observed of almost all compounds, except for dodecyl-*N*-[2-(*N,N,N'*-trimethylammonio)ethyl] maleamate methylsulfate (**16**) (Fig. 14). The use of eluents with $\text{pH} > 5.0$ containing buffer solutions is not appropriate, since the peaks of compounds separate too late under such conditions, and they are asymmetric. These undesirable effects can be reduced, if the concentration of organic modifier and buffer solution is increased in mobile phase.

Separation selectivity is strongly influenced by the differences in hydrophobicity of compounds. The substitution of dodecylgroup by benzylgroup of maleic acid reduces k of all studied pH values, as a result the selectivity of the pair *N*-dodecyl-[2-(*N,N,N'*-trimethylammonio)ethyl]diamide 4-methylbenzenesulfonate (**12**) and *N*-benzyl-[2-(*N,N,N'*-trimethylammonio)ethyl]diamide 4-methylbenzenesulfonate of maleic acid (**13**) is larger than 2.

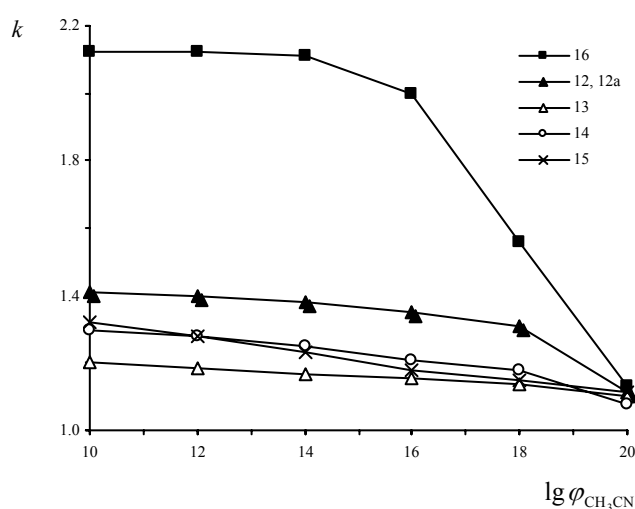


Fig. 14. Dependence of retention factor on the $\lg \varphi_{\text{CH}_3\text{CN}}$ for the maleic acid derivatives of quaternary diamides (**12** – **16**) investigated ($\text{pH} = 4.0$).

The effectiveness of column *CM300* ($6\mu\text{m}$, $100 \times 4.6\text{ mm}$) against the studied compounds ranges from 200 to 2900 theoretical plates and depends on the composition of the mobile phase. A higher effectiveness is observed at lower pH of mobile phase and increased concentrations of organic modifier (Fig. 15).

The values of the peak tailing factors range from 1.1 to 3.8. These values tend to decrease when pH of mobile phase increases.

In the chromatography of the studied quaternary maleic acid derivatives RP ion-pair method should be regarded as the most appropriate method. By using this method symmetric peaks and higher effectiveness are obtained, as compared to the ion-exchange method.

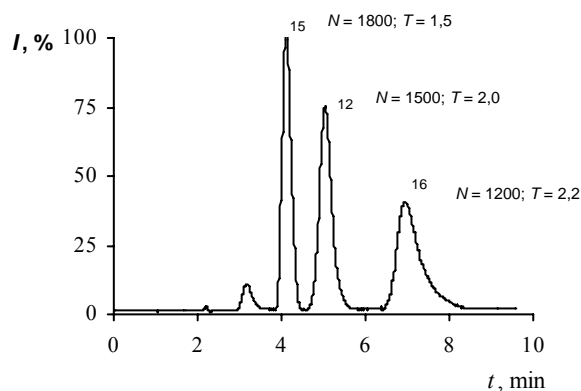


Fig. 15. Chromatogram of quaternary maleic acid ammonium derivatives (**12**, **15** and **16**) on the carboxymethyl weak cation exchanger (CM300 WAX) with 10% $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ 0.001 M sodium citrate solution ($\text{pH}=4.0$) as the mobile phase.

CONCLUSIONS

1. Oligomers of the alkyl(polytrihydroxypropyl) maleates can be separated on the silica gel as a stationary phase and gradient of the propan-2-ol/n-hexane/diethyl ether (5/35/60 \rightarrow 40/0/60) as the mobile one during 40 min. Formation of the trimethylsilyl derivatives after treatment with *N,O*-bis-trimethylsilyltrifluoroacetamide is a convenient method for the separation of alkyl(poly-2-hydroxypropyloxy)maleates and alkyl(poly-1-hydroxymethylethyloxy) derivatives on capillary column with the subsequent identification by mass spectrometry.

2. Diamides of the maleic acid can be analyzed on the octadecylsilica gel as a stationary phase at high content (>70%) of CH_3OH and CH_3CN water solutions as the mobile ones. Efficiency of such chromatographic systems characterize by 3000 – 20000 theor.plates/meter. The associate's formation between sorbate and organic modifier together with adsorption-desorption equilibria in the monomolecular layer of sorbent has been demonstrated is possible.

3. Quaternary maleic acid ammonium derivatives can be analyzed on the octadecylsilica/methanol/phosphate buffer system. The increase of the retention factor k with the decrease of CH₃OH content in the eluent can be observed at all pH values investigated. The efficiency of the system depends on the concentration of organic modifier and pH of the buffer solution. The retention time depends on the nature of the hydrophobic substituents.

4. Analysis of the quaternary maleic acid derivatives can be performed by the ion pair mode using system octadecylsilica/methanol/citrate buffer solution with the sodium hexanesulfonate as the ion pair agent. Linear relation exists between $\lg k$ and concentration of the organic modifier.

5. Analysis of the quaternary maleic acid derivatives is possible performed on the weak carboxymethyl cation exchanger (*CM 300 WAX*) as the stationary phase and 10-20% CH₃CN/0,001 M sodium citrate in the pH range 3.5-5.5. Retention parameters decrease with the increase of the mobile phase pH and CH₃CN content. The efficiency of chromatographic system investigated is about 200-29000 theor.plates/meter.

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