

SEPARATION PROCESSES
AT THE INTERFACES. CHROMATOGRAPHY

**The Structural Effect of Some 1,3,4-Oxadiazoles
and 1,2,4,5-Tetrazines on Their Thermodynamic Characteristics
of Adsorption from Aqueous Organic Solutions
on Phenyl-Bonded Silica Gel**

B. R. Saifutdinov, N. A. Gorbunov, and A. A. Pimerzin

Samara State Technical University, Samara, 443100 Russia

e-mail: sayf_br@mail.ru

Received April 28, 2014

Abstract—The thermodynamic characteristics of adsorption of substituted 1,3,4-oxadiazoles and 1,2,4,5-tetrazines from aqueous organic solutions on phenyl-bonded silica gel were determined in the Henry region via the method of high-performance liquid chromatography. The influence of the structure of the studied compounds on their thermodynamic characteristics of adsorption from binary aqueous organic solvents of different natures was examined. The correlation dependences of the standard differential molar changes in the Gibbs energy, enthalpy, and entropy of adsorption on the van der Waals surface area and reduced dipole moment of molecules of the examined compounds were found. The main factors in optimization of the separation techniques and adjustment of the adsorption selectivity of phenyl-bonded silica gel were determined under conditions of reversed-phase high-performance liquid chromatography.

DOI: 10.1134/S2070205114060185

INTRODUCTION

The reversed-phase liquid chromatography method with aqueous organic mobile phases and non-polar adsorbents is the most widespread technique of liquid chromatography in current practice [1, 2]. Adsorbents grafted with groups containing aromatic fragments have begun to be used alongside the classical silica gels grafted with alkyl groups [3]. The simplest example of such stationary phases is the silica gels grafted with phenyl groups with spacers of various natures and sizes [4]. The peculiarities of these materials are due to the electron-donating properties of their surface because of its π basicity, with the strength of the latter changing depending on the surface concentration of grafted groups and the length of spacer [1]. After the adsorption of aromatic molecules or molecules containing multiple bonds on these stationary phases, π – π interactions between adsorbate and adsorbent are considered to be occurred [3, 4]. This circumstance explains the π selectivity of the sorbents providing the separation of such important compounds as tetracyclines, anesthetics, narcotic analgesics, benzodiazepines, nucleosides, and compounds containing nitro, azido, or sulfonyl groups [5]. Other features of phenyl-containing adsorbents consist in the possibility of eluting the mixtures of highly polar compounds with 100% aqueous mobile phases and performing hydrophilic chromatography with the use of solvents with a high content of organic components [5]. Moreover, a higher selectivity of methanol as a

mobile phase component in comparison to acetonitrile has been reported in the case of the separation by means of phenyl-containing adsorbents [5].

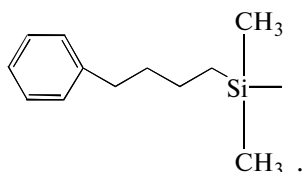
Silica gel grafted with dimethyl-(4-phenyl-*n*-butyl)silyl groups was previously characterized under conditions of high-performance liquid chromatography (HPLC) [6, 7]. The adsorption properties of this silica gel were examined on the basis of a study of adsorption thermodynamics and did not confirm the presence of π – π interactions between the adsorbate and adsorbent, which have been strongly manifested, for example, in HPLC with the use of hyper-cross-linked polystyrene [8]. The obtained data, in contrast, indicated great similarity of adsorption properties of the investigated stationary phase and octadecyl silica gel [9]. To optimize the techniques for the separation of mixtures on silica gels grafted with phenyl groups and control the adsorption selectivity, it is important to understand whether π – π interactions between adsorbate and adsorbent take place during HPLC on phenyl-containing adsorbents and to estimate their contribution to retention. The solution to this problem seems to consist in disclosure of regularities of the influence of key factors determining adsorption selectivity on the thermodynamic characteristics of adsorption (TCA) of test compounds on such adsorbents. The structure of adsorbates is likely to be one of the main factors [10–12].

The aim of the work is to find out the dependences of TCA of substituted 1,3,4-oxadiazoles and 1,2,4,5-tetrazines from aqueous organic solutions on silica gel

grafted with dimethyl-(4-phenyl-*n*-butyl)silyl groups on the structure of their molecules.

EXPERIMENTAL

The instruments, chromatographic column, and experimental conditions have been described in detail in [6, 7]. The experiment was performed with the use of a Prominence liquid chromatograph (Shimadzu, Japan) equipped with an LC-20AD semipreparative gradient pump, a DGU-20A₃ mobile-phase degasser, an SPD-20A spectrophotometric detector with a thermostatically controlled cell, and a CTO-20A column thermostat. The commercially available Ascentis[®] Phenyl silica gel (Supelco Analytical, United States) chemically modified with dimethyl-(4-phenyl-*n*-butyl)silyl groups was used as an adsorbent, with the structure of grafted group being as follows:



The size of adsorbent particles was 5 μm , the average pore diameter was 100 \AA , the surface concentration of grafted groups was 2.44 groups nm^{-2} , the dimensions of the chromatographic column were 2.1 \times 150 mm, specific surface area $S_{\text{sp}} = 450 \text{ m}^2 \text{ g}^{-1}$, and mass of adsorbent in the column $g = 0.26 \text{ g}$. Column temperature T was varied within a range from 313.15 to 333.15 K in steps of 5 K.

Aryl-, hetaryl-, and cyclohexyl-substituted 1,3,4-oxadiazoles and 1,2,4,5-tetrazines were employed as adsorbates. Their structures and purity were confirmed by data of elemental analysis; mass spectrometry; and IR, ¹H NMR, and ¹³C NMR spectroscopy, as well as with the use of chromatography–mass spectrometry.

The elution in HPLC was fulfilled in an isocratic mode with aqueous acetonitrile (MeCN–H₂O), methanol (MeOH–H₂O), and isopropanol (*i*-PrOH–H₂O) solvents of 1 : 1 (vol) ratio at a flow rate of the mobile phase of 500 $\mu\text{L min}^{-1}$.

On the basis of the primary chromatographic data, the following TCA from solutions were calculated. Thus, the standard differential molar change in Gibbs energy of adsorption ($\Delta_a G^\circ$, kJ mol^{-1}) was calculated based on distribution constant K_c at 313.15 K via the equation [13]

$$\Delta_a G^\circ = -RT \ln(K_c/K_{c,\text{st}}),$$

where $K_c = kV_M/v_a + 1$ (k is the retention factor); V_M , μL , is the volume of the mobile phase in the column measured by the system peak [2]; v_a , μL , is the volume of the adsorption layer ($v_a = 88 \mu\text{L}$) [14, 15]; and

$K_{c,\text{st}}$ is the distribution constant in the standard state ($K_{c,\text{st}} = 1$) [6, 7].

To determine $\ln K_c$ at different temperatures of the chromatographic column, the standard differential molar changes in enthalpy ($\Delta_a H^\circ$, kJ mol^{-1}) and entropy ($\Delta_a S^\circ$, J (mol K)^{-1}) of adsorption were calculated via the linear regression analysis method with the use of the following equation [6, 13]:

$$\ln K_c = -\Delta_a H^\circ/RT + \Delta_a S^\circ/R. \quad (1)$$

The techniques for the calculation of lipophilicity ($\log P$), van der Waals surface area (S_w , \AA^2), dipole moment (μ , D), and polarizability (α , \AA^3) of adsorbate molecules have been described in [16].

RESULTS AND DISCUSSION

Along with other factors, the structure of adsorbate molecules is known to play a key role in the selectivity of HPLC separation due to the differences in geometric and electronic structures of analytes, determining the energy of intermolecular interactions between adsorbate and adsorbent and those between adsorbate and bulk solution. The effect of differences in the structures of studied compounds on their retention on silica gel grafted with dimethyl-(4-phenyl-*n*-butyl)silyl groups is manifested in the coefficients of equation relating the logarithm of retention factor to the inverse temperature of column, namely, $\ln k = B/T + A$ (Table 1). The corresponding values of the coefficients of determination represented in Table 1 show that the temperature dependence of retention is well linearized in the Van't Hoff equation coordinates.

The data in Table 1 indicate that, in the case of adsorption from both aqueous acetonitrile and aqueous alcohol solutions, the values of coefficient $B = -\Delta_a H^\circ/R$ depend on the nature of heterocycles. The highest values of B coefficient are typical of nonpolar 1,2,4,5-tetrazines (compounds **15–17**), likely due to the presence of hydrophobic interactions in the bulk solution leading to the expulsion of molecules containing large nonpolar fragments from the polar medium onto the nonpolar surface of silica gel grafted with phenyl groups [17]. Among other heterocycles, the high B values are also observed in the cases of bromo-containing 1,3,4-oxadiazoles (compounds **12–14**) because of large values of polarizability of their molecules [16]. At the adsorption from aqueous acetonitrile solution, the B value of 2-[di(thienyl-2,2')-5]-5-cyclohexyl-1,3,4-oxadiazole (compound **4**) is comparable with those of brominated 1,3,4-oxadiazoles; from isopropanol solution, the intermediate between those of bromo-containing 1,3,4-oxadiazoles and 1,2,4,5-tetrazines; and from aqueous methanol solution, the highest. As this coefficient is proportional to the change in enthalpy of adsorption, then the differences between its values of the examined substances indicate differences between energies of the processes

Table 1. The equation coefficients of the dependence of the logarithm of retention factor on the inverse temperature of the column $\ln k = BT^{-1} + A$ at the adsorption of studied heterocyclic compounds from aqueous organic solutions on silica gel grafted with phenyl groups

Entry	Adsorbate	MeCN-H ₂ O (1 : 1)			<i>i</i> -PrOH-H ₂ O (1 : 1)			MeOH-H ₂ O (1 : 1)		
		<i>B</i>	- <i>A</i>	<i>r</i> ²	<i>B</i>	- <i>A</i>	<i>r</i> ²	<i>B</i>	- <i>A</i>	<i>r</i> ²
1	2,5-Diphenyl-1,3,4-oxadiazole	1.1089	2.1034	0.989	1.5773	3.9008	0.994	3.3306	7.1431	1.000
2	2-(Thienyl-2)-5-phenyl-1,3,4-oxadiazole	1.1052	2.1953	1.000	1.5845	4.0681	1.000	3.2897	7.2974	1.000
3	2-(Thienyl-2)-5-cyclohexyl-1,3,4-oxadiazole	1.0000	1.6400	1.000	1.5334	3.7246	0.998	3.2423	6.7190	1.000
4	2-[Di(thienyl-2,2')-5]-5-cyclohexyl-1,3,4-oxadiazole	1.2638	1.3428	1.000	2.2271	4.9169	1.000	4.7507	9.2126	1.000
5	2,5-Di(thienyl-2)-1,3,4-oxadiazole	1.1052	2.3053	1.000	1.4181	3.6516	0.997	3.0041	6.6519	1.000
6	2-(4-Nitrophenyl)-5-(thienyl-2)-1,3,4-oxadiazole	1.3155	2.6459	1.000	2.1769	5.5770	0.998	3.5515	7.8212	1.000
7	2-Phenyl-5-(4-fluorophenyl)-1,3,4-oxadiazole	1.2103	2.3306	1.000	1.7149	4.2342	1.000	3.3581	7.1028	1.000
8	2-(Thienyl-2)-5-(4-fluorophenyl)-1,3,4-oxadiazole	1.1568	2.2685	0.999	1.6378	4.0473	1.000	3.1585	6.7106	1.000
9	2-(Selenophenyl-2)-5-(4-fluorophenyl)-1,3,4-oxadiazole	1.2103	2.3506	1.000	1.7006	4.1717	1.000	3.2358	6.8094	1.000
10	2-Phenyl-5-(4-chlorophenyl)-1,3,4-oxadiazole	1.1568	1.7885	0.999	1.9847	4.7110	1.000	3.7533	7.6863	1.000
11	2-(Thienyl-2)-5-(4-chlorophenyl)-1,3,4-oxadiazole	1.2085	2.0516	0.997	1.8434	4.3355	1.000	3.5314	7.2237	1.000
12	2-(4-Bromophenyl)-5-phenyl-1,3,4-oxadiazole	1.2085	1.8316	0.997	2.0269	4.7140	1.000	3.9923	8.1960	1.000
13	2-(4-Bromophenyl)-5-(thienyl-2)-1,3,4-oxadiazole	1.1568	1.7685	0.999	1.9956	4.6793	1.000	3.7504	7.6848	1.000
14	2-(4-Bromophenyl)-5-(selenophenyl-2)-1,3,4-oxadiazole	1.2620	2.0138	0.999	2.0175	4.6679	0.999	3.7946	7.6748	1.000
15	3,6-Diphenyl-1,2,4,5-tetrazine	1.5258	2.7666	1.000	2.5055	5.8301	1.000	4.2710	8.8541	1.000
16	3,6-Di(thienyl-2)-1,2,4,5-tetrazine	1.5258	2.7666	1.000	2.4389	5.7918	0.999	3.9928	8.4117	1.000
17	3,6-Di(selenophenyl-2)-1,2,4,5-tetrazine	1.6273	2.8338	0.993	2.5908	6.0128	1.000	4.3087	8.9345	1.000

*r*² is the coefficient of determination of the $\ln k$ vs. T^{-1} dependence. The numbers of adsorbates given in the table are the same as in the body of the article and figures.

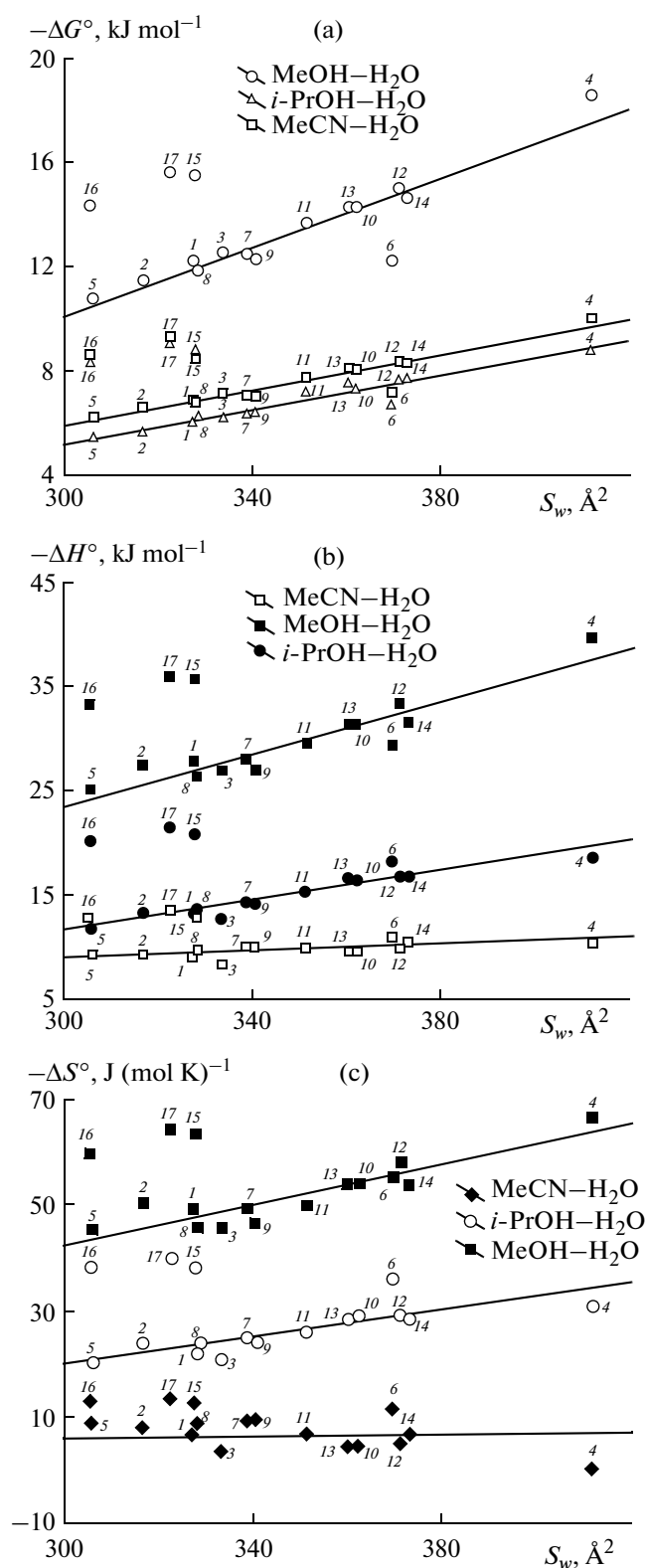


Fig. 1. The dependences of the standard differential molar changes in (a) Gibbs energy, (b) enthalpy, and (c) entropy of adsorption of 1,3,4-oxadiazoles and 1,2,4,5-tetrazines from aqueous organic solutions on silica gel grafted with phenyl groups on the van der Waals surface area of their molecules.

accompanying their adsorption under the HPLC conditions.

The nature of adsorbate also strongly influences the A coefficient, which is proportional to the change in entropy of adsorption (Table 1). In the cases of adsorption from aqueous acetonitrile and isopropanol solutions, the maximum values of this coefficient are observed for nonpolar 1,2,4,5-tetrazines. This result is due to a strong delocalization of their molecules on the surface caused, in turn, by hydrophobic interactions in the associated bulk solution [17]. In comparison to other adsorbates, 2-(4-nitrophenyl)-5-(thienyl-2)-1,3,4-oxadiazole (compound **6**) is characterized by higher A values of adsorption from aqueous acetonitrile and isopropanol solutions. In the case of adsorption from aqueous methanol solution, 2-[di(thienyl-2,2')-5]-5-cyclohexyl-1,3,4-oxadiazole (compound **4**) demonstrates the maximum A value.

In addition, the data in Table 1 indicate a significant influence of the nature of organic component of the mobile phase on both energy and entropy components of the change in Gibbs energy of adsorption. The B and A coefficients increase after the transition from aqueous acetonitrile to aqueous isopropanol and then to aqueous methanol solution. Their values appear to be affected by such factors as the particularities of adsorbate solvation by the organic component of the mobile phase in the bulk solution and those of adsorption of the organic component on the surface of the stationary phase [7]. Solvation of the studied heterocycles seems to increase after successive transitions from aqueous methanol solution to aqueous isopropanol and acetonitrile ones [7], as the lipophilicity of the organic components of eluents decreases after the transition from acetonitrile ($\log P = 0.65$) to isopropanol ($\log P = 0.49$) and methanol ($\log P = -0.27$). Because of the presence of large hydrophobic moieties in the molecules of investigated compounds, they should be more solvated by more lipophilic molecules, thus resulting in the reduction of absolute values of effective changes in enthalpy and entropy. The higher the content of acetonitrile in the adsorption layer, in comparison to methanol, the lower the absolute values of effective changes in enthalpy and entropy. This circumstance is due to the increased energy costs for the expulsion of organic component of eluent from adsorption layer and to the release of the greater number of degrees of freedom after its expulsion into the bulk solution in the case of acetonitrile in comparison to methanol [7].

Figure 1a shows the dependences of the standard differential molar changes in Gibbs energy of adsorption of examined compounds from aqueous organic solutions on silica gel grafted with phenyl groups on the van der Waals surface area of these compounds. The data in this figure, as well as in Table 1, indicate that all adsorbates are adsorbed from aqueous methanol solution to a significantly greater extent than from aqueous acetonitrile solution and even more

than from aqueous isopropanol one. The use of all compositions of mobile phases leads to the deviation of the points corresponding to 1,2,4,5-tetrazines (compounds **15**–**17**) toward higher $-\Delta_a G^\circ$ values from the linear $|\Delta_a G^\circ|$ versus S_w dependences typical of the majority of 1,3,4-oxadiazoles. The strong adsorption of nonpolar 1,2,4,5-tetrazines is likely due to the hydrophobic interactions in the associated solutions of the mobile phases [17] leading to the expulsion of molecules containing large nonpolar moieties onto the nonpolar surface of adsorbent. In the case of adsorption from both aqueous alcohol and acetonitrile solutions, the points corresponding to nitro-substituted adsorbate (compound **6**), in contrast, deviate from the linear $-\Delta_a G^\circ$ versus S_w dependences toward lower $|\Delta_a G^\circ|$ values. This result seems to be due to the higher polarity of molecules of this compound ($\mu = 6.011$ D) in comparison to other 1,3,4-oxadiazoles [16], resulting in higher energy of intermolecular interaction with components of the bulk solution and, thus, in a decrease in adsorption from the solutions.

In general, the retention of studied heterocyclic compounds agrees well with the known regularities of reversed-phase HPLC, namely, in the case of the majority of 1,3,4-oxadiazoles, the $|\Delta_a G^\circ|$ values increase symbatically with S_w , regardless of the nature of organic component of the solution from which adsorption occurs (Fig. 1a). It is of interest to note the somewhat stronger adsorption of cyclohexyl-substituted 1,3,4-oxadiazole (compound **3**) from aqueous organic solutions in comparison to its closest analogue such as 2-(thienyl-2)-5-phenyl-1,3,4-oxadiazole (compound **2**). In the case of adsorption of these heterocycles on hyper-cross-linked polystyrene characterized by the presence of π – π interactions between adsorbate and adsorbent, the opposite pattern occurs, namely, a decrease in adsorption is observed after the introduction of cyclohexyl substituent instead of phenyl group [8]. The substitution of phenyl group by cyclohexyl one enhances the adsorption from aqueous organic media on the classic reversed-phase material, such as octadecyl silica gel because of the conformational flexibility of cyclohexyl moiety and its possibility to penetrate into the conformationally mobile grafted layer [16]. The regularities of adsorption of studied compounds on silica gel grafted with phenyl groups are similar to the features of adsorption on octadecyl silica gel under the conditions of reversed-phase HPLC and significantly different from those of the adsorption on hyper-cross-linked polystyrene. Thus, adsorption and chromatographic data represented in the form of $\Delta_a G^\circ$ indicate that the selectivity of separation under the conditions of reversed-phase HPLC on silica gel grafted with dimethyl-(4-phenyl-*n*-butyl)silyl groups is strongly influenced by the dispersion interactions (as in the case of octadecyl silica gel) rather than π – π interactions between adsorbate and adsorbent. The lower content of carbon on the surface of phenyl-containing silica in comparison to

octadecyl silica gel results in the weaker adsorption of the same compounds on this adsorbent [6, 16]. This fact also indicates the dominant role of dispersion interactions between adsorbate and adsorbent. In the case of HPLC on silica gel grafted with phenyl groups, the key role of these interactions is confirmed by the linear $-\Delta_a G^\circ$ versus S_w dependence typical of the most 1,3,4-oxadiazoles. The leaning of phenyl groups against the silica substrate due to the contact with polar medium, as well as the π stacking between adjacent grafted phenyl groups, have been proposed as possible reasons for the lack of the π selectivity of the examined adsorbent [7].

However, interpretation of enthalpy and entropy contributions to the adsorption of investigated heterocycles on silica gel grafted with phenyl groups discloses a complex mechanism of processes accompanying HPLC on this adsorbent. Analysis of the data in Figs. 1b, 1c makes it possible to find out the effects responsible for the mentioned above regularity, that is, for the stronger adsorption of cyclohexyl-containing heterocycle (compound **3**) on the studied adsorbent in contrast to its phenyl-containing analogue (compound **2**). Thus, Fig. 1b shows the higher absolute value of the change in enthalpy of adsorption of 2-(thienyl-2)-5-phenyl-1,3,4-oxadiazole from aqueous organic solutions than that of 2-(thienyl-2)-5-cyclohexyl-1,3,4-oxadiazole. Moreover, the data in Fig. 1c indicate the larger absolute value of the change in entropy of adsorption of 2-(thienyl-2)-5-phenyl-1,3,4-oxadiazole than in the case of 2-(thienyl-2)-5-cyclohexyl-1,3,4-oxadiazole. In other words, the $|\Delta_a G^\circ|$ value of cyclohexyl-containing adsorbate (compound **3**) is higher than that of its phenyl-containing analogue (compound **2**), while the latter exhibits higher values of $|\Delta_a H^\circ|$ and $|\Delta_a S^\circ|$ (Fig. 1). In the case of 2-(thienyl-2)-5-phenyl-1,3,4-oxadiazole (compound **2**), the energy of π – π interactions between the adsorbate and adsorbent is likely to be higher than in the case of 2-(thienyl-2)-5-cyclohexyl-1,3,4-oxadiazole (compound **3**). As a result, the phenyl-containing heterocycle is characterized by larger $|\Delta_a H^\circ|$ values (Fig. 1b). At the same time, in the case of adsorption from aqueous organic solutions of various nature, an increased conformational flexibility of cyclohexyl moiety in comparison to phenyl group seems to result in higher values of $|\Delta_a S^\circ|$ of 2-(thienyl-2)-5-cyclohexyl-1,3,4-oxadiazole (compound **3**) in contrast to those of 2-(thienyl-2)-5-phenyl-1,3,4-oxadiazole (compound **2**) (Fig. 1c). Therefore, an increased adsorption on silica gel grafted with phenyl groups, manifested in larger $|\Delta_a G^\circ|$ values, is observed in the case of cyclohexyl-containing heterocycle (Fig. 1a). This result may be explained by the mutual compensation of enthalpy contribution responsible for π – π interaction between adsorbate and adsorbent and the entropy contribution responsible for the conformational flexibility of the cyclohexyl fragment. The competition between these two factors seems to determine the $|\Delta_a G^\circ|$ values measured in the

Table 2. The equation coefficients of the dependences of the TCA of studied heterocyclic compounds from aqueous organic solutions on silica gel grafted with phenyl groups on the van der Waals surface area of their molecules

Bulk solution	$-\Delta G^\circ = a + bS_w$			$-\Delta H^\circ = a + bS_w$			$-\Delta S^\circ = a + bS_w$		
	$-a$	b	r^2	$-a$	b	r^2	$-a$	b	r^2
MeCN–H ₂ O (1 : 1)	5.0141	0.0363	0.980	–4.0273	0.0164	0.455	–26.1225	0.0546	0.281
<i>i</i> -PrOH–H ₂ O (1 : 1)	5.2121	0.0346	0.978	10.2687	0.0726	0.890	18.1048	0.1276	0.652
MeOH–H ₂ O (1 : 1)	11.5048	0.0717	0.972	14.4694	0.1259	0.879	14.6625	0.1901	0.816

r^2 is the coefficient of determination of the dependence of corresponding TCA on S_w .

adsorption experiment and, hence, the chromatographic behavior of compounds. Thereby, the increased adsorption of cyclohexyl-substituted heterocycle is likely to indicate, not the lack of π – π interactions between adsorbate and adsorbent during HPLC on silica gel grafted with phenyl groups, but the fact that the enthalpy contribution induced by these interactions is offset by the entropy contribution caused by the conformational flexibility of cyclohexyl moiety. As a result, the presence of π – π interactions between adsorbate and adsorbent does not lead to π selectivity of silica gel grafted with phenyl groups, as this contribution is not enough to suppress the conformational effects occurred during the adsorption on chemically modified silicas [1].

Figure 1b also shows that the increase in $|\Delta_a H^\circ|$ is symbiotic with S_w values. This result is typical of the majority of adsorbates, with the only exception being nonpolar 1,2,4,5-tetrazines exhibiting the deviation of corresponding point toward larger $|\Delta_a H^\circ|$ values. An increase in the enthalpy of adsorption after the enhancement of the van der Waals surface area of molecules is due to the gain caused by an enlargement of the contact area between adsorbate and adsorbent and by an increase in the energy of dispersion interactions. In general, these dependences confirm the known principles of reversed-phase HPLC. Another interesting fact should also be mentioned. It has been noted above that the $|\Delta_a G^\circ|$ values of all compounds are significantly reduced after the transition from an aqueous methanol solution to an aqueous acetonitrile one and reduced then less significantly after the transition to aqueous isopropanol solution (Fig. 1a). On the other hand, the transition from aqueous methanol solution to aqueous isopropanol and acetonitrile ones results in a sharp decrease in the $|\Delta_a H^\circ|$ values of all adsorbates (Fig. 1b). In the same manner, the change in entropy of adsorption ($|\Delta_a S^\circ|$) depends on the nature of organic component of the bulk solution (Fig. 1c). In other words, the $|\Delta_a H^\circ|$ and $|\Delta_a S^\circ|$ values of examined compounds are lower in the case of adsorption from aqueous acetonitrile solution than from aqueous isopropanol one, while the $|\Delta_a G^\circ|$ value demonstrates the opposite pattern. The higher $|\Delta_a H^\circ|$ and $|\Delta_a S^\circ|$ values of adsorption from aqueous isopropanol solution are likely to be due to the increased solvation of adsorbate

molecules in aqueous acetonitrile media and the high content of acetonitrile in adsorption layer [7]. Perhaps, in the case of adsorption from aqueous acetonitrile solutions, the enthalpy and entropy components of Gibbs energy diminish to varying degrees due to the influence of these factors. Therefore, the $|\Delta_a G^\circ|$ value determined as a difference between these components [18–20] is higher in the case of adsorption from aqueous acetonitrile media in comparison to aqueous isopropanol ones. Thereby, the investigated substances are adsorbed to a greater extent from aqueous acetonitrile solutions (Fig. 1a).

It is important to note that the enhancement of the van der Waals surface area of molecules leads to an increase in not only $|\Delta_a G^\circ|$ and $|\Delta_a H^\circ|$ (Figs. 1a, 1b), but also $|\Delta_a S^\circ|$ values (Fig. 1c). In general, the $-\Delta_a G^\circ$ versus S_w dependences of 1,3,4-oxadiazoles are linear in the case of adsorption from aqueous organic solutions of different nature. As hydrophobic interactions influence not only the energy component but also entropy, the points corresponding to nonpolar 1,2,4,5-tetrazines, like those of the dependences of Gibbs energy and enthalpy, deviate from the linearity toward higher values [17]. The increase in the absolute value of the change in entropy after the enhancement of S_w seems to be due to the stronger localization of molecules of a larger size on the surface. Thus, the dependences of TCA from solutions correlate with the surface area of molecules. The equation coefficients of the dependence of TCA on S_w and the corresponding coefficients of determination are given in Table 2. The data indicate the best correlation of Gibbs energy with the van der Waals surface area of molecules, whereas the correlations of enthalpy and entropy are somewhat worse. As to enthalpy and entropy, the correlations between the TCA and S_w are more accurate in the case of aqueous alcohol solutions, which is likely due to the stronger association of these solvents and, consequently, the higher localization of adsorbate molecules during adsorption from them. One can also see from Fig. 1 that the selectivity of separation of adsorbates in aqueous methanol and isopropanol mobile phases is higher than in aqueous acetonitrile ones. This result can be explained by the maximum difference in TCA of the pairs of compounds which occurs during the adsorption from aqueous methanol solutions. That is,

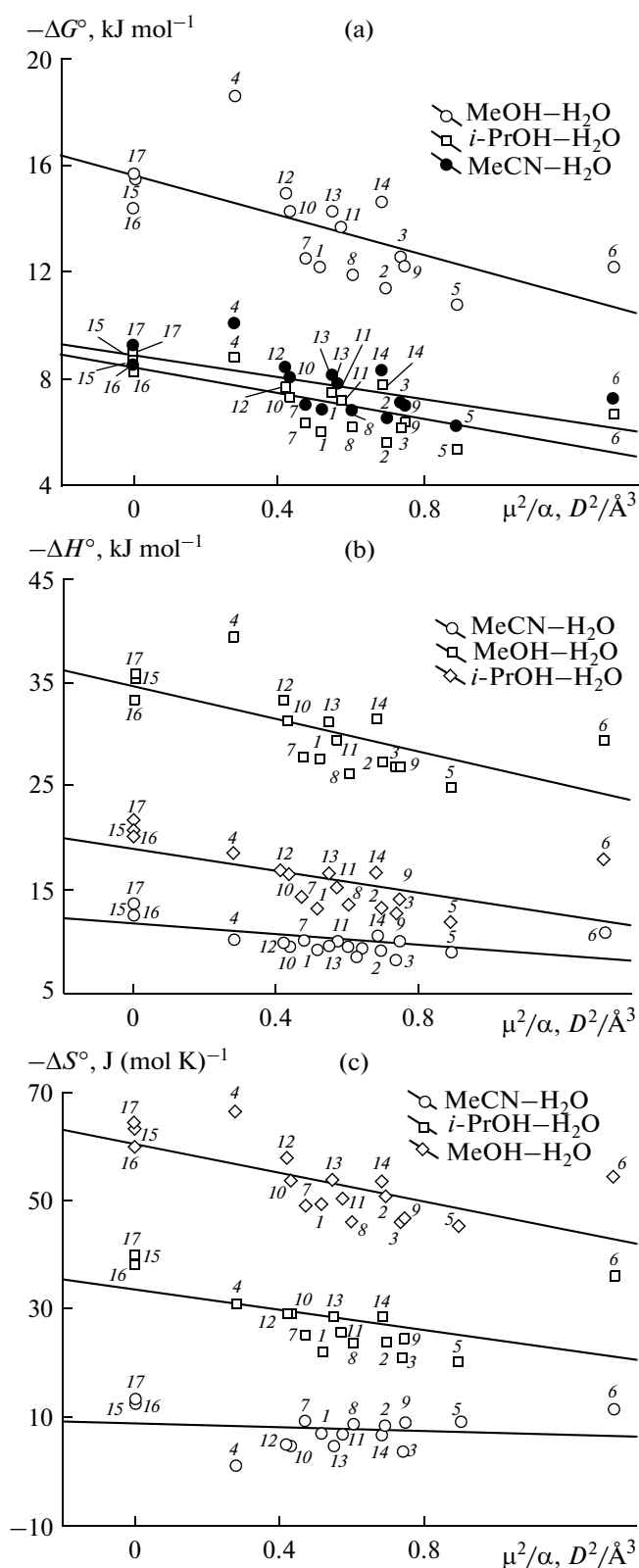


Fig. 2. The dependences of the standard differential molar changes in (a) Gibbs energy, (b) enthalpy, and (c) entropy of adsorption of 1,3,4-oxadiazoles and 1,2,4,5-tetrazines from aqueous organic solutions on silica gel grafted with phenyl groups on the reduced dipole moment of their molecules.

the outcomes of the present study confirm the data declared by the manufacturer on the higher selectivity of separation on silica gel grafted with phenyl groups with the use of methanol, rather than acetonitrile, as a component of the mobile phase [5]. However, the high selectivity of separation of investigated compounds with methanol is considerably restricted by much higher adsorption of the compounds from aqueous-methanol media in comparison to the adsorption from aqueous acetonitrile solutions (Fig. 1), leading to reduction of separation efficiency.

The dependences of the standard differential molar changes in (a) Gibbs energy, (b) enthalpy, and (c) entropy of adsorption of examined compounds from aqueous organic solutions on the reduced dipole moment of their molecules are shown in Fig. 2. The enthalpies of adsorption from solutions have been previously shown [9] to depend on the reduced dipole moment, which is the most appropriate measure of the polarity of the bulk molecules [21, 22]. Under the conditions of reversed-phase HPLC, the retention characteristics, as a rule, do not correlate directly with the dipole moment of molecules [23], whereas the use of the reduced dipole moment makes it possible to describe the dependences of TCAs from solutions on the polarity of adsorbates. Figure 2 indicates the reduction of the absolute values of all TCAs after an increase in the reduced dipole moment of adsorbates. In the case of adsorption under the conditions of reversed-phase HPLC, this result is obviously due to the essential role of intermolecular dipole-dipole interactions of adsorbate with the components of the bulk solution of mobile phase. The strong deviation of the point corresponding to cyclohexyl-containing adsorbate (compound 4) from the dependence typical of most heterocycles is likely to be owing to the size of its molecule comprising, in contrast to other studied substances, four cycles.

CONCLUSIONS

The examination of the dependence of thermodynamic characteristics of adsorption of 1,3,4-oxadiazoles and 1,2,4,5-tetrazines from aqueous organic solutions on silica gel grafted with dimethyl-(4-phenyl-*n*-butyl)silyl groups showed that the π selectivity of studied adsorbent is significantly restricted by the conformational flexibility of grafted groups and molecules of adsorbates. In terms of the π selectivity, the investigated stationary phase is inferior to hyper-cross-linked polystyrene and exhibits chromatographic behavior similar to that of octadecyl silica gel. In this case, the adsorption of investigated compounds by phenyl-containing silica is weaker than that by octadecyl silica gel.

It was found that the nature of organic component of the mobile phase significantly influences the thermodynamic characteristics of adsorption of 1,3,4-oxadiazoles and 1,2,4,5-tetrazines. After the transition from aqueous acetonitrile and aqueous isopro-

panol solutions to methanol ones, the selectivity of separation of investigated compounds increases with a simultaneous substantial increase in adsorption.

Thus, the use of silica gel grafted with dimethyl-(4-phenyl-*n*-butyl)silyl groups along with aqueous acetonitrile mobile phases is optimal for HPLC to separate the compounds strongly retained on octadecyl silica gel.

ACKNOWLEDGMENTS

We thank Dr. A.V. Yudashkin, assistant professor of the Organic Chemistry Department, Samara State Technical University, for kindly providing the synthesized samples of heterocyclic compounds.

In the research, the facilities of the Study of Physical and Chemical Properties of Substances and Materials Center for Collective Use of the Samara State Technical University were used.

This work was supported by the Ministry of Education and Science of Russian Federation, the basic part of a state assignment, project no. 2014/199.

REFERENCES

- Lisichkin, G.V., Fadeev, A.Yu., Serdan, A.A., et al., *Khimiya privitykh poverkhnostnykh soedinenii* (Chemistry of Grafted Surface Compounds), Lisichkin, G.V., Ed., Moscow: Fizmatlit, 2003.
- Sadek, P., *The HPLC Solvent Guide*, New York: Wiley, 2002.
- Horak, J., Maier, N.M., and Lindner, W., *J. Chromatogr. A*, 2004, vol. 1045, p. 43.
- Chan, F., Yeung, L.S., LoBrutto, R., and Kazakevich, Y.V., *J. Chromatogr. A*, 2005, vol. 1069, p. 217.
- Supelco Catalog*, HPLC Chapter, 2013, p. 18.
- Saifutdinov, B.R. and Pimerzin, A.A., *Russ. J. Phys. Chem. A*, 2013, vol. 87, p. 529.
- Saifutdinov, B.R. and Pimerzin, A.A., *Russ. J. Phys. Chem. A*, 2013, vol. 87, p. 2013.
- Saifutdinov, B.R., Davankov, V.A., and Il'in, M.M., *Russ. J. Phys. Chem. A*, 2014, vol. 88, p. 358.
- Saifutdinov, B.R., Kurbatova, S.V., and Emel'yanova, N.S., *Russ. J. Phys. Chem. A*, 2010, vol. 84, p. 673.
- Reinhard, M. and Drefahl, A., *Handbook for estimating physicochemical properties of organic compounds*, New York: Wiley, 1999.
- Kaliszan, R., Wiczling, P., Markuszewski, M.J., and Al-Haj, M.A., *J. Chromatogr. A*, 2011, vol. 1218, p. 5120.
- Belyakova, L.D., Buryak, A.K., and Larionov, O.G., *Prot. Met. Phys. Chem. Surf.*, 2013, vol. 49, p. 605.
- Liu, Y., *J. Chem. Eng. Data*, 2009, vol. 54, no. 7, p. 1981.
- El'tekov, Yu.A., *Zh. Fiz. Khim.*, 1991, vol. 65, p. 2573.
- Saifutdinov, B.R., *Russ. J. Phys. Chem. A*, 2013, vol. 87, p. 512.
- Kurbatova, S.V., Saifutdinov, B.R., Larionov, O.G., and Meshkovaya, V.V., *Russ. J. Phys. Chem. A*, 2009, vol. 83, p. 471.
- Israelachvili, J.N., *Intermolecular and Surface Forces*, Oxford: Elsevier, 1991.
- Parfitt, G.D. and Rochester, C.H., *Adsorption from Solution at the Solid/Liquid Interface*, London: Academic, 1983.
- Tani, K. and Suzuki, Y., *Chromatographia*, 1991, vol. 31, p. 347.
- Guillaume, Y.C. and Guinchard, C., *Anal. Chem.*, 1996, vol. 68, p. 2869.
- Ioffe, B.V., *Refraktometricheskie metody v khimii* (Refractometric Methods in Chemistry), Leningrad: Khimiya, 1983.
- Solomonov, B.N. and Konovalov, A.I., *Russ. Chem. Rev.*, 1991, vol. 60, p. 25.
- Dolgonosov, A.M., *Nespetsificheskaya selektivnost' v probleme modelirovaniya vysokoeffektivnoi khromatografii* (Non-specific Selectivity in the Modeling of High Performance Chromatography), Moscow: Librokom, 2013.

Translated by D. Lonshakov