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ORIGINAL PAPER

Influence of mono- and two-component organic modifiers on determination of lipophilicity of tetradentate Schiff bases

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Abstract

The influences of the application of mono- and two-component organic modifiers on lipophilicity determination of 12 tetradentate Schiff bases by reversed-phase thin layer chromatography were investigated. The main goal is to estimate types of interaction between observed compounds and components of the applied chromatographic systems and establish some behaviour pattern in order to easier choose a combination of organic modifiers which will simulate interaction in biological systems based on the facts that the same basic intermolecular interactions are responsible for the behaviour of substances in both the biological and chromatographic system. The applied organic modifier shows the ability to modify the surface of the applied sorbent, which affects the manifestation of lipophilicity of the observed compounds. Mono-component organic modifiers from different groups of the Snyder triangle were used, as well as their two-component mixtures. In addition, we compared experimentally determined calculated parameters of lipophilicity.

Keywords Schiff base · Lipophilicity · RP-TLC · Organic modifiers

Introduction

Lipophilicity is a physico-chemical characteristic of a compound that is related to the passive passage of the compound through biological membranes, as well as their solubility. It also affects the establishment of interactions between the biologically active compound and the active site of the receptor. The biological activity of a molecule is conditioned by the processes of its pharmacokinetics (the path from the place of drug administration to the place of action) and its pharmacodynamics, i.e. specifics of the action itself. These two processes depend on the behaviour of the molecules in the non-aqueous (cell membrane) and aqueous medium (the cell interior) (Hansch and Fujita 1964; Henchoz et al. 2009; Hill and Young 2010; Waring 2009). The experimental methods for determination of lipophilicity have been classified into direct ("shake-flask"), indirect (chromatographic, spectrophotometric, optical, electrochemical, etc.), and calculation methods (specialized software using mathematical models) (Starek et al. 2021). The conventional "shake-flask" procedures where the soluble concentration in each phase of the equilibrated water–immiscible organic mixture is determined by spectrophotometric or chromatographic methods are time-consuming, limited in terms of pH range, and intended to be used on extremely pure compounds (Sangster 1997). Nowadays, this approach has been almost completely substituted by modern chromatographic techniques, mainly directed by adsorption and partitioning processes (Henchoz et al. 2008; Hiroshi 1986; Paneth et al. 2017; Starek et al. 2013).

Reversed phase thin layer chromatography (RP-TLC) is widely used for the determination of lipophilicity due to the fact that the same basic intermolecular interactions are responsible for the behaviour of substances in both the biological and chromatographic systems (Brzezińska and Koška 2006; Perušković et al. 2015; Sławik and Paw 2003). The organic modifier, as a component of the mobile phase of the chromatographic system, achieves specific interactions with the stationary phase, as well as with the analytes molecules (Zapała and Waksmundzka-Hajnos 2005). Adequate selection of an organic modifier is crucial for the rapid, efficient,

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and reliable determination of lipophilicity of various classes of compounds in order to predict their behaviour in a real biological system, such as passage through a cell membrane. In addition to the basic characteristics of the organic modifier, such as various parameters that express polarity, the viscosity is also important, which is responsible for reducing the dispersion during the chromatographic separation process (Krasikov 2003). The solvent's acidity and alkalinity define the ability of organic modifiers to act as proton donors or proton acceptors when building hydrogen bonds with appropriate sorbents. The molecules of the mobile phase (water and organic modifier) are partially sorbed on the surface of the stationary phase in the process of chromatographic separation. This process in RP systems is focused only on the competitive reaction of the organic modifier with the analyte molecules, due to the nonpolar surface of the stationary phase, and therefore, the applied organic modifier is responsible for differences in stationary phase properties due to changes in the organic component in the mobile phase. Cserhati et al. found significant differences using paraffin-impregnated silica gel, depending on the organic modifier used (methanol, acetone, or acetonitrile) (Cserháti, 1984).

Increased concentration of the organic component in the mobile phase leads to a decrease in the retention of analyte molecules (Anderson et al. 1997; Blagus et al. 2010). Elution power, as one of the most important characteristics of the solvent used in chromatography, largely depends on the sorbent used and is a measure of its capacity to enter intermolecular interactions, i.e. represents the adsorption energy of analytes molecules per unit area of sorbent. The elution power of the solvent mixture is calculated based on the elution power of each individual solvent and their share in the mixture. It is necessary that the mobile phase has the appropriate elution power to obtain optimal values of retention parameters (R_F values in the range 0.2–0.8). The experimentally obtained retention parameters (R_M) directly depend on the concentration of the organic phase in the chromatographic system (Anderson et al. 1997). By extrapolation to the content of organic solvent in the mobile phase of 0%, the lipophilicity parameter $R_M{}^0$ was obtained. Linear correlation can be expressed by equation $R_M = R_M{}^0 + m\varphi$, where φ is concentration of organic compound in mobile phase, while m is slope and represents the hydrophobic surface of the test compound (Baošić et al. 2007; Brzezińska and Koška 2006).

In our laboratory, we investigate tetradentate Schiff bases with confirmed biological activity for many years (Aburas et al. 2012, 2013; Baošić et al. 2007, 2008; Baošić et al., 2003; Perušković et al. 2015, 2020; Stevanović et al. 2017). These investigations gave us the opportunity to form a model system that can be used for investigation and estimation of different effects that will depend on the structure of the Schiff bases themselves. Schiff bases may contain a variety of substituents with different electron-donating or electron-withdrawing groups, and have interesting properties such as catalytic activity, ability to reversibly bind oxygen, transfer of amino group, antibacterial, and antifungicidal activities (Dugas and Penney 1981; Jones et al. 1979; Mohamed 2006; Olive and Olive 1984; Raman et al. 2003; Singh and Varshney 2006).

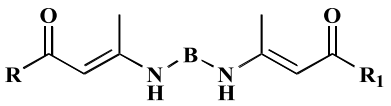
The aim of this work is to investigate the effect of the application of mono and two-component organic modifiers in lipophilicity determination by RP-TLC in order to estimate types of interaction between observed compounds and components of the applied chromatographic systems and establish some behaviour pattern in order to easier choose a combination of organic modifiers which simulate interaction in biological systems. In addition, we compared experimentally determined parameters of lipophilicity and calculated values.

Experimental

Investigated compounds

Investigation is performed on the set of 12 tetradentate Schiff bases derivates of β-diketones and diamines (Table 1). They contain etan-1,2-diamine or propane-1,2-diamine as amin part and pentane-2,4-dion and/or 1-phenylbutane-1,3-dione, pentane-2,4-dione and/or 1,1,1-trifluoropentane-2,4-dione and 1,1,1-trifluoro-pentane-2,4-dione

Table 1 Investigated tetradentate Schiff bases



No	Schiff base	R	R_1	B
(1)	H_2 (*acac*$_2$ en)[a]	CH_3	CH_3	CH_2CH_2
(2)	H_2 (*acac phacac*en)[b]	CH_3	C_6H_5	CH_2CH_2
(3)	H_2 (*phacac*$_2$en)	C_6H_5	C_6H_5	CH_2CH_2
(4)	H_2 (*phacac tfacac*en)[c]	C_6H_5	CF_3	CH_2CH_2
(5)	H_2 (*acac tfacac*en)	CH_3	CF_3	CH_2CH_2
(6)	H_2 (*tfacac*$_2$en)	CF_3	CF_3	CH_2CH_2
(7)	H_2 (*acac*$_2$pn)[d]	CH_3	CH_3	$CH(CH_3)CH_2$
(8)	H_2 (*acac phacac*pn)	CH_3	C_6H_5	$CH(CH_3)CH_2$
(9)	H_2 (*phacac*$_2$pn)	C_6H_5	C_6H_5	$CH(CH_3)CH_2$
(10)	H_2 (*phacac tfacac*pn)	C_6H_5	CF_3	$CH(CH_3)CH_2$
(11)	H_2 (*acac tfacac*pn)	CH_3	CF_3	$CH(CH_3)CH_2$
(12)	H_2 (*tfacac*$_2$pn)	CF_3	CF_3	$CH(CH_3)CH_2$

[a]*acac* = pentane-2,4-dione, en = etan-1,2-diamine

[b]*phacac* = 1-phenilbutane-1,3-dione

[c]*tfacac* = 1,1,1-trifluoropentane-2,4-dione

[d]pn = propane-1,2-diamine

and/or 1-phenylbutane-1,3-dione. All compounds prepared according to previously reported procedures (Baošić et al. 2003; McCarthy et al. 1955).

Chromatography

Commercially available RP-18 F_{254} silica gel plates (Merck, Darmstadt, Germany), size 10 × 10 cm, were used. Compositions of applied mono- and two-component organic modifiers in mobile phases are listed in Table 2. Five mobile phases representing a mixture of an organic modifier (1–5) and water in the following volume ratios of 8:2, 6:4, 4:2 and 2:8 was used. In addition, four mobile phases were applied, which in the organic part of the mobile phase contained a mixture of two organic solvents (6–9), i.e. modifiers which are mixed with water in a volume ratio of 8:2, 6:4, 4:2 and 2:8. 1.0 µL of freshly prepared solutions of Schiff bases, at a concentration of 5 mg/mL, were applied to the plates.

After drying the applied zones, chromatographic development was performed in a horizontal chromatography chamber (Camag horizontal HPTLC development chamber in the tank configuration). Prior to development, the plates were equilibrated with solvent vapour for 30 min. All solvents used were of analytical purity. After chromatogram development, the zones of the examined Schiff bases were detected using a UV lamp. The obtained retention parameters represent the mean value of the three measurements. All measurements were made at room temperature (22 ± 2 °C).

Calculations

The calculation of the lipophilicity parameters examined Schiff bases was done in the software package Schrodinger Suite 2017-1 (Schrödinger Release 2017-1: Schrödinger, LLC, New York, NY 2017). Molecular structures were generated using Maestro 11.1. Conformational analysis was then performed using Conformational Search from the Macromodel V11.6 module. The force field OPLS-2005 was used for the conformational analysis, and as a water solvent. The generated structures are energetically minimized by the Polak-Ribiere conjugate gradient with a maximum of 2500 steps. Structures were purified by discarding repetitive ones. The molecular descriptors of the test compounds were determined using the QikProp module from the Schrödinger Suite program. To determine the descriptors, the best conformational structures of the compounds were used. The following parameters have been determined: hexadecane/gas partition coefficient ($logPC_{16}$), octanol/gas partition coefficient ($logP_{oct}$), water/gas partition coefficient ($logP_w$), octanol/water partition coefficient ($logP_{o/w}$), solubility in water (logS), solubility in water conformation undependent (CIlogS), IC_{50} values for blocking of HERG K^+ chanal (logHERG), predicted observable Caco cell permeability (PCaco), predicted brain/blood partition coefficient (logBB), predicted observable MDCK cell permeability (PMDCK), predicted skin permeability (logKp), bonding to human serum albumine (log K_{HSA}), human oral absorption (HOA), values of Lipinski five rules (Ruleof Five), values of Jorgensena three rules (RuleOfThree). Statistical calculations and Pearsons correlations were done using the NCSS statistical package (Hintze 2001). Bate-Smith and Westall equation was used for calculation of R_M values (Bate-Smith and Westall 1950).

Table 2 Composition of applied organic modifiers

No	Organic modifiers
1	Methanole (MeOH)
2	Tetrahydrofurane (THF)
3	Acetonitrile (ACN)
4	Acetone (Ac)
5	*n*-Propanole (*n*-PrOH)
6	Acetonitrile: methanole (1:1)
7	Acetonitrile: tetrahydrofurane (1:1)
8	Acetonitrile: acetone (1:1)
9	Methanole: tetrahydrofurane (1:1)

Results and discussion

Determination of lipophilicity

To investigate the effect of the organic modifier and their mixtures on the experimental determination of lipophilicity parameters, Schiff bases were chromatographed under reverse-phase thin layer chromatography (RP-TLC) condition. The mobile phase in reverse-phase thin layer chromatography is most often a mixture of water and an organic modifier. Organic modifiers of different characteristics that define the behaviour in the reverse-phase chromatographic system were selected, in order to examine their effect on the determination of lipophilicity parameters of the tested compounds. The polarity of the organic modifier is usually the most important for its selection as a component of the mobile phase and represents the ability of the solvent to realize interactions with the analyte (dipole–dipole interactions or polarization). Table 3 shows the characteristics of the applied organic modifiers (Lide 2004; Rudakov and Sedishev 2003; Snyder 1974).

The chromatographic behaviour of the investigated compounds, in addition to non-specific interactions with the stationary phase, is also influenced by specific interactions with the mobile phase (Apostolov et al. 2020). Different strength of interaction of the investigated compounds with the stationary or mobile phase, when using different organic

Table 3 Some characteristics of applied organic modifiers

Organic modifier	ε	D	S	P	χ_d	χ_e	χ_n	η
THF	7.5	1.75	4.4	4.0	0.38	0.20	0.42	0.46
n-PrOH	22.8	1.68	4.1	4.0	0.54	0.19	0.27	2.30
Ac	21.5	2.88	3.4	5.1	0.35	0.23	0.42	0.32
MeOH	31.2	1.70	3.0	5.1	0.48	0.22	0.31	0.60
ACN	37.1	3.92	3.1	5.8	0.31	0.27	0.42	0.37

ε—dielectric constant; D—dipol moment; S—elution power for RP chromatographic system; P—Snyder polarity parameter; χ_e—proton acceptor contribution; χ_d—proton donor contribution; χ_n—dipol interaction contribution; η-viscosity (mPa s)

solvents, will affect the differences in R_M values, and thus the $R_M{}^0$ values as measure of lipophilicity. The applied organic modifiers have different proton-donor and proton-acceptor abilities, as well as a tendency towards dipole interactions, depending on the type and structure of the observed Schiff bases.

The retention of the tested compounds decreases with the amount of organic component in the mobile phase, which improves the performance of the mobile phase and gives an opportunity for extrapolation of the composition of the mobile phase to pure water. Investigated set of compounds belong to homologous series, applied as a model system for investigation of the effect of substituents, effect of diamine bridge structure on chromatographic behaviour, as well as on experimental determination of lipophilicity parameters. Applied mobile phases prepared as mixture of one or two organic modifiers and water represent a chromatographic model system in which the influence of the organic mobile phase part on the experimental determination of lipophilicity parameters of the observed Schiff bases was investigated.

This approach can provide significant information about the behaviour of observed Schiff bases in applied chromatographic systems in which the various effects that an organic modifier may have on the entire molecule of the test compound or on individual parts thereof are expressed. Based on this information, it is possible to define the specific type of organic modifiers which would simulate the real surrounding during to transport through the cell membrane. The lipophilicity parameters $R_M{}^0$ obtained using various organic modifiers, together with statistical parameters, are shown in Table 4.

The absolute values of the slope of the curves obtained by correlating the retention parameter R_M and the fraction of the organic modifier in the mobile phase (i.e. its concentrations) are listed in Table 5.

Influence of organic modifiers

The obtained results confirm the fact that organic modifiers have influence on the determination of lipophilicity parameters. Different values of lipophilicity parameters for the same compounds were obtained by using different organic solvents as modifiers. Comparing the characteristics of the used organic modifiers, as well as their mixtures, the most significant differences exist in the elution power, proton-donor, and proton acceptor abilities. The elution power of the organic modifier represents its ability to dissolve the compounds, while the selectivity of the organic modifier is characterized by parameters that define its proton-donor, proton-acceptor properties, as well as interactions with dipoles.

The highest slope values are obtained when THF were used as organic modifier, while the lowest slope were obtained with MeOH (Table 5). This is in line with the increase in the elution power of these organic modifiers. In relation to all applied organic modifiers, THF has the highest (4.4), while MeOH has the lowest elution power (3.0) (Rudakov and Sedishev 2003). The elution power increases with increasing concentration of the organic modifier in the mobile phase. These two solvents are quite different in other properties. Methanol is compared to THF proton-donor and does not achieve significant interactions with the stationary RP phase, while THF tends to be adsorbed by non-specific interactions on the surface of the sorbent, which may have a positive effect on determining lipophilicity of compounds. The lipophilic properties of the investigated Schiff bases that would correspond to those they have in a real environment, e.g. when passing through the cell membrane. Expressed sorption of THF, where a monolayer of organic modifier is formed on the surface of the stationary phase, so that the polar part of the THF molecule is turned towards the surface, i.e. towards the Schiff base, which enables stronger interactions of the compound with the stationary phase surface and thus leads to stronger retention. The sorption mode on the sorbent surface is different for different organic modifiers. The THF and ACN form a thin multimolecular layer on a stationary phase, while MeOH is adsorbed in the form of a monomolecular layer. This leads to a fundamentally large difference in the retention mechanism of the Schiff bases in the applied hydro-organic chromatographic systems.

The retention of the compounds is a consequence of the distribution in three different processes: the analyte is distributed between an organic modifier mixed with water in

Table 4 Lipophilicity and statistical parameters obtained with different organic modifiers

No	MeOH				Ac				THF			
	R_M^0	*m*	*r*	*s*	R_M^0	*m*	*r*	*s*	R_M^0	*m*	*r*	*s*
1	0.606	− 1.341	0.958	0.155	0.503	− 1.106	0.975	0.090	0.880	− 2.060	0.987	0.132
2	1.598	− 2.268	0.978	0.188	0.797	− 1.616	0.993	0.075	1.153	− 1.788	0.998	0.041
3	1.443	− 2.114	0.997	0.059	1.268	− 2.102	0.983	0.153	1.265	− 1.528	0.996	0.055
4	1.273	− 1.682	0.946	0.224	1.032	− 2.232	0.991	0.115	1.527	− 2.235	0.974	0.200
5	0.854	− 1.802	0.991	0.094	0.733	− 1.550	0.989	0.089	1.677	− 2.600	0.999	0.037
6	1.305	− 1.934	0.975	0.169	1.498	− 2.492	0.971	0.237	1.586	− 2.449	0.993	0.112
7	0.625	− 1.217	0.990	− 0.193	0.472	− 1.102	0.976	0.095	1.062	− 2.164	0.978	0.180
8	1.001	− 1.781	0.971	0.170	1.083	− 1.941	0.976	0.168	1.141	− 2.342	0.984	0.162
9	1.324	− 1.510	0.992	0.076	1.069	− 2.002	0.961	0.224	1.454	− 2.474	0.996	0.091
10	1.425	− 1.941	0.986	0.128	1.083	− 1.941	0.976	0.168	1.443	− 2.482	0.982	0.187
11	0.807	− 1.434	0.964	0.154	0.997	− 1.958	0.988	0.120	1.193	− 2.269	0.995	0.084
12	1.620	− 2.034	0.997	0.061	1.213	− 2.381	0.972	0.222	1.766	− 2.833	0.991	0.145
No	ACN				*n*-PrOH				ACN/MeOH			
	R_M^0	*m*	*r*	*s*	R_M^0	*m*	*r*	*s*	R_M^0	*m*	*r*	*s*
1	0.844	− 1.669	0.983	0.122	0.247	− 1.179	0.971	0.112	0.740	− 1.521	0.993	0.068
2	1.546	− 2.392	0.985	0.160	1.312	− 2.400	0.954	0.293	1.321	− 2.079	0.994	0.086
3	1.523	− 2.604	0.995	0.099	1.101	− 2.212	0.974	0.199	1.422	− 1.670	0.979	− 1.521
4	1.423	− 2.314	0.992	0.116	1.003	− 1.853	0.993	0.083	1.056	− 1.840	0.994	0.077
5	1.660	− 3.044	0.989	0.173	0.813	− 1.773	0.977	0.150	1.272	− 2.109	0.978	0.174
6	1.834	− 3.655	0.984	0.255	1.523	− 2.801	0.998	0.062	1.530	− 2.320	0.991	0.118
7	1.229	− 2.050	0.997	0.059	0.750	− 1.488	0.952	0.185	0.683	− 1.240	0.946	0.165
8	0.779	− 1.450	0.989	0.083	0.474	− 1.848	0.988	0.111	0.745	− 1.361	0.986	0.090
9	1.463	− 2.470	0.991	0.126	1.138	− 2.345	1.000	0.025	1.262	− 2.088	0.932	0.315
10	1.429	− 2.040	0.999	0.035	1.186	− 2.026	0.987	0.130	1.174	− 1.679	0.987	0.107
11	1.532	− 2.400	0.985	0.165	1.405	− 2.502	0.997	0.070	2.525	− 3.828	0.956	0.457
12	1.326	− 2.309	0.999	0.037	1.158	− 2.600	0.973	0.240	0.984	− 1.703	0.938	0.244
No	MeOH/THF				ACN/THF				ACN/Ac			
	R_M^0	*m*	*r*	*s*	R_M^0	*m*	*r*	*s*	R_M^0	*m*	*r*	*s*
1	0.536	− 1.144	0.934	0.170	0.408	− 1.185	0.964	0.127	0.765	− 1.586	0.996	0.056
2	1.002	− 1.752	0.977	0.147	0.910	− 1.708	0.966	0.177	0.962	− 1.536	0.976	0.132
3	1.575	− 2.482	0.982	0.184	1.025	− 2.021	0.990	0.112	1.325	− 2.061	0.977	0.173
4	1.063	− 1.828	0.987	0.115	1.336	− 2.222	0.992	0.110	1.045	− 1.802	0.990	0.099
5	1.260	− 2.137	0.990	0.117	0.959	− 1.975	0.997	0.060	2.463	− 3.880	0.951	0.490
6	1.533	− 2.214	0.967	0.226	1.318	− 2.369	0.996	0.087	1.656	− 2.430	0.998	0.055
7	0.582	− 1.258	0.985	0.086	0.840	− 1.406	0.999	0.021	0.880	− 1.791	0.999	0.032
8	0.840	− 1.421	0.979	0.114	0.664	− 1.504	0.994	0.062	1.064	− 1.622	0.995	0.062
9	1.044	− 1.772	0.997	0.051	1.326	− 1.949	0.908	0.349	1.240	− 2.017	0.999	0.037
10	1.141	− 1.982	0.946	0.262	0.955	− 1.740	0.992	0.087	1.273	− 2.110	0.985	0.144
11	0.683	− 1.436	0.930	0.220	0.856	− 1.593	0.991	0.083	0.935	− 1.830	0.995	0.071
12	1.157	− 1.801	0.968	0.181	1.109	− 1.767	0.965	0.185	1.376	− 2.031	1.000	0.012

the mobile phase, an organic modifier located in the stationary phase and the surface of the modified sorbent. The establishment of equilibrium in these three processes leads to different behaviour of the compounds in the applied chromatographic systems depending on the properties of the organic modifier in mobile phase. These equilibria directly affect the expression of the lipophilicity properties of the observed compounds and give the possibility of finding optimal organic modifiers, i.e. optimal chromatographic systems in which the determination of lipophilicity would correspond

Table 5 Slope values (m) as measure of hydrophobicity of investigated compounds (1–12)

	MeOH	Ac	THF	ACN	*n*-PrOH	ACN/MeOH	MeOH/THF	ACN/THF	ACN/Ac
1	− 1.341	− 1.106	− 2.060	− 1.669	− 1.179	− 1.521	− 1.144	− 1.185	− 1.586
2	− 2.268	− 1.616	− 1.788	− 2.392	− 2.400	− 2.079	− 1.752	− 1.708	− 1.536
3	− 2.114	− 2.102	− 1.528	− 2.604	− 2.212	− 1.670	− 2.482	− 2.021	− 2.061
4	− 1.682	− 2.232	− 2.235	− 2.314	− 1.853	− 1.840	− 1.828	− 2.222	− 1.802
5	− 1.802	− 1.550	− 2.600	− 3.044	− 1.773	− 2.109	− 2.137	− 1.975	− 3.880
6	− 1.934	− 2.492	− 2.449	− 3.655	− 2.801	− 2.320	− 2.214	− 2.369	− 2.430
7	− 1.217	− 1.102	− 2.164	− 2.050	− 1.488	− 1.240	− 1.258	− 1.406	− 1.791
8	− 1.781	− 1.941	− 2.342	− 1.450	− 1.848	− 1.361	− 1.421	− 1.504	− 1.622
9	− 1.510	− 2.002	− 2.474	− 2.470	− 2.345	− 2.088	− 1.772	− 1.949	− 2.017
10	− 1.941	− 1.941	− 2.482	− 2.040	− 2.026	− 1.679	− 1.982	− 1.740	− 2.110
11	− 1.434	− 1.958	− 2.269	− 2.400	− 2.502	− 3.828	− 1.436	− 1.593	− 1.830
12	− 2.034	− 2.381	− 2.833	− 2.309	− 2.600	− 1.703	− 1.801	− 1.767	− 2.031

to the real environment, whereby the obtained values would be valid for the continuation of the examination of biological activity.

In order to determine the influence of the nature of the organic modifier on the experimental determination of lipophilicity parameters, $R_M{}^0$, the Pearson correlation matrix of parameters obtained by applying the mentioned mono- and two-component organic modifiers was observed (Table 6). This correlation can provide further explanation of the similarities and dissimilarities of the applied organic modifiers that are manifested in the interactions with the molecules of the tested compounds, as well as with the surface of the stationary phase. Estimation of the types of interaction between observed compounds and components of the applied chromatographic systems and determination of some behaviour pattern give possibilities for choosing a combination of organic modifiers, whether applied to test compounds and their derivatives or to structurally similar ones. Such selected condition will simulate interaction in biological systems based on the facts that the same basic intermolecular interactions are responsible for the behaviour of substances in both the biological and chromatographic system.

The correlation coefficients indicate that there are clearly defined differences between the organic modifiers used, which are the result of the properties they can exhibit in the chromatographic system when interacting with the stationary phase and with the tested compounds. This means that in the applied RP chromatographic system, the influence of the type of applied organic modifier on the determination of lipophilicity parameters is expressed. In the case of monocomponent organic modifiers, the best correlation was found between Ac and MeOH, regardless of the significant difference in elution power (Ac 3.4; MeOH 3.0) and the fact that MeOH is protic, while Ac is an aprotic solvent.

Namely, the values of their polarity parameters P, defined by Snyder, as well as the values of the contribution χe (proton acceptor contribution), χd (proton donor contribution) and χn (contribution dipole interaction) are remarkably close, which results in very similar behaviour in the observed reverse-phase system and the realization of similar interactions in type and intensity, both with the surface of the sorbent and with the tested compounds. On the other hand, THF with lower polarity but stronger elution power in the RP chromatographic system shows a satisfactory correlation with Ac and ACN. Also, the high correlation obtained using *n*-PrOH and ACN is unexpected, given their large difference in individual properties, such as polarity parameter and position in Snyder's eluotropic series. It is likely that by exhibiting their properties, these organic modifiers achieve interactions that are opposite in effect. Thus, *n*-PrOH

Table 6 Pearson correlation matrix of $R_M{}^0$ values obtained by applying different mono- and two-component organic modifiers

	MeOH	Ac	THF	ACN	*n*-PrOH	ACN/MeOH	MeOH/THF
Ac	0.7044						
THF	0.5367	0.6307					
ACN	0.4228	0.5722	0.6005				
n-PrOH	0.6208	0.7276	0.5018	0.8483			
ACN/MeOH	0.0843	0.4280	0.1368	0.6163	0.7016		
MeOH/THF	0.6759	0.7956	0.6816	0.6713	0.5412	0.1923	
ACN/THF	0.6069	0.7138	0.7603	0.7211	0.7039	0.2460	0.6557
ACN/Ac	0.1259	0.2895	0.7242	0.5515	0.1868	0.1155	0.6507

achieves stronger interactions with the stationary phase, while ACN favours interactions with the tested compounds. In this way, they lead to a similar effect. Alcohol, by its interactions with the stationary phase, increases its polarity, while ACN decreases the polarity of the tested compounds. The data given in Table 6 show that two-component organic modifiers show a satisfactory correlation with organic modifiers, whether mono- or two-component, only in relation to the values of elution power they exhibit.

Experimetally obtained lipophilicity vs. calculated

Experimentally determined lipophilicity parameters of the tested set of tetradentate Schiff bases, were correlated with calculated lipophilicity parameters in order to consider the effect of organic modifier on the experimental determination of lipophilicity and their agreement to calculated values. It is known that there is a statistically significant correlation between retention parameters obtained by reverse-phase chromatography and computer-calculated lipophilicity parameters (Hawrył et al. 2015). The obtained Pearson correlation matrix is shown in Table 7.

The obtained correlation coefficients indicate a significant dependence of experimentally determined lipophilicity parameters on the applied organic modifier, which significantly affects changes in the monolayer on the surface of RP sorbent, the orientation of molecules of tested compounds in the mobile phase, and their interaction in each chromatographic system.

The correlation coefficients of lipophilicity parameters indicate the fact that the use of THF, as an organic modifier, enables such a chromatographic system in which are favoured interactions for which the lipophilicity of the compound itself is solely responsible. The lipophilicity parameter $R_M{}^0$ (THF) shows the highest correlation in the Pearson correlation matrix (0.782) with logPoct (predicted octanol/gas partition coefficient), as well as with logPo/w (predicted octanol/water partition coefficient) (0.654), which confirms that THF is a modifier of choice for use in the RP chromatographic system in determining lipophilicity parameters of test or structurally similar compounds. The highest negative correlation coefficient (− 0.788) is shown with logS (predicted solubility in water). The lipophilicity parameter $R_M{}^0$ (THF) is in accordance with logBB, i.e. predicted brain/blood partition coefficient and PMDCK parameter predicting passage through the cell membrane.

Such a good connection with the calculated parameters of lipophilicity, namely those that define the passage through the cell membrane, i.e. allow better distribution in the body, shows the parameter of lipophilicity experimentally obtained in the presence of acetone and methanol as an organic modifier. The use of two-component organic modifiers, regardless of the obtained lipophilicity parameters, does not show a significant correlation with the calculated one, except for the mixture ACN/THF, probably because the individual components of the mixture have high elution power and aprotic properties. Two-component organic modifiers increase the number of interactions to which the test compounds may be exposed, as well as the surface area of the stationary phase. In this way, the number of equilibria in which the tested compound participates increases, and it cannot express its lipophilicity, which corresponds to that in the real environment. The use of two-component organic modifiers, regardless of the obtained lipophilicity parameters, does not show a significant correlation with the calculated one. Also, the components of the binary organic modifier compete on the surface of the sorbent, which most likely does not achieve the uniformity of the stationary phase.

Conclusions

The lipophilicity parameters of the series of tetradentate Schiff bases were determined under reverse-phase chromatography conditions using various mono- and two-component organic modifiers. From the obtained results, the influence of the applied organic modifier on the chromatographic behaviour of the tested compounds and on the determination

Table 7 Correlation matrix of experimental and calculated lipophilicity parameters

	logPC16	logPoct	logPw	logPo/w	logS	CIlogS	logHERG	PCaco	logBB	PMDCK	logKp[a]
MeOH	0.411	0.741	0.525	0.719	− 0.711	− 0.794	− 0.545	0.353	0.250	0.400	0.552
THF	0.172	0.782	0.273	0.654	− 0.685	− 0.788	− 0.275	0.464	0.564	0.533	0.383
Ac	− 0.109	0.547	0.031	0.447	− 0.600	− 0.556	− 0.143	0.326	0.679	0.644	0.089
*n*PrOH	− 0.055	0.430	0.050	0.253	− 0.328	− 0.377	− 0.107	0.197	0.395	0.239	0.100
ACN/MeOH	0.043	0.561	0.129	0.468	− 0.562	− 0.537	− 0.247	0.472	0.508	0.408	0.256
MeOH/THF	− 0.084	0.236	− 0.067	0.149	− 0.193	− 0.187	0.018	0.340	0.345	0.125	0.063
ACN/THF	0.203	0.658	0.314	0.478	− 0.431	− 0.673	− 0.202	0.131	0.313	0.296	0.326
ACN/Ac	0.259	0.779	0.382	0.665	− 0.743	− 0.723	− 0.507	0.450	0.398	0.354	0.449

[a]Abbreviations are given in experimental part

of lipophilicity parameters was determined, as well as that by applying a certain modifier the observed molecule can show its lipophilicity. In different organic modifiers, the interactions of Schiff base with the stationary and with the mobile phase are different. In addition, the applied organic modifier shows the ability to modify the surface of the applied sorbent, which affects the manifestation of lipophilicity of the observed compounds. The use of MeOH as an organic modifier allows the best separation of structurally similar compounds. However, due to the low correlation between the obtained R_M retention parameters and the methanol concentration, this is not suitable for determining lipophilicity. In addition, MeOH forms a monolayer on the surface of the sorbent, which completely modifies its surface and makes it difficult to express the lipophilicity of the observed molecules. In contrast, the use of THF leads to the fact that the tested compounds can be considered as a homologous series. Accordingly, THF is a recommended organic mobile phase modifier suitable for determining the lipophilicity of test compounds using reverse phase thin layer chromatography. The established regularity in chromatographic behaviour and in the procedure of determining lipophilicity parameters, as well as the connection with the calculated lipophilicity parameters, suggests the possibility of choosing the target chromatographic system for Schiff bases and their derivatives as well as for structurally similar compounds which will simulate interaction in biological systems.

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Declarations

Conflict of interest The authors declare that they have no conflict of interest.

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