Characterization of Ascentis RP-Amide column: Lipophilicity measurement and linear solvation energy relationships

Deborah Benhaim, Eli Grushka*

Institute of Chemistry, The Hebrew University of Jerusalem, Edmund Safra Campus, Jerusalem 91904, Israel

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ABSTRACT

This study investigates lipophilicity determination by chromatographic measurements using the polar embedded Ascentis RP-Amide stationary phase. As a new generation of amide-functionalized silica stationary phase, the Ascentis RP-Amide column is evaluated as a possible substitution to the *n*-octanol/water partitioning system for lipophilicity measurements. For this evaluation, extrapolated retention factors, log k'_w, of a set of diverse compounds were determined using different methanol contents in the mobile phase. The use of *n*-octanol enriched mobile phase enhances the relationship between the slope (S) of the extrapolation lines and the extrapolated log k'_w (the intercept of the extrapolation), as well as the correlation between log P values and the extrapolated log k'_w (1:1 correlation, $r^2 = 0.966$). In addition, the use of isocratic retention factors, at 40% methanol in the mobile phase, provides a rapid tool for lipophilicity determination. The intermolecular interactions that contribute to the retention process in the Ascentis RP-Amide phase are characterized using the solvation parameter model of Abraham. The LSER system constants for the column are very similar to the LSER constants of the *n*-octanol/water extraction system. Tanaka radar plots are used for quick visual comparison of the system constants of the Ascentis RP-Amide column and the *n*-octanol/water extraction system. The results all indicate that the Ascentis RP-Amide stationary phase can provide reliable lipophilic data.

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1. Introduction

Lipophilicity of xenobiotics is one of the key parameters to be examined at an early stage of drug research. Lipophilicity is required for the prediction of the absorption, distribution, metabolism and excretion (ADME) properties as well as for drug pharmacokinetics and pharmacodynamics [1–3].

Usually, lipophilicity is expressed by the logarithm of the partition coefficient of a neutral compound in *n*-octanol/water system (log P) or by the logarithm of the distribution coefficient for an ionized compound (log D^{pH}). Both log P and log D have been widely incorporated as physicochemical descriptors for quantitative structure–activity relationships (QSARs) or for quantitative structure–retention relationships (QSRRs) [4–8].

Due to the several disadvantages in the determination of *n*-octanol/water partition coefficients by the shake-flask method (emulsion problems, large amount of pure compounds required, tedious, time consuming, etc.), the chromatographic retention process, especially reversed-phase HPLC (RP-HPLC), is being used for this purpose. A judicious choice of chromatographic separation

system provides a suitable model for estimating *n*-octanol/water partition coefficients [9–13]. Using a standard set of solutes, a correlation model (QSRR), shown in Eq. (1), is constructed between known log P or log D values and chromatographic retention data, chiefly log k', of the solutes, obtained for a given mobile phase and stationary phase system. The correlation is defined by Eq. (1):

$$\log P \text{ or } \log D = a + b \log k' \tag{1}$$

The logarithm of the isocratic retention factor, log k', as well as the logarithm of an extrapolated retention factor to 100% water, log k'_w, are defined as chromatographic lipophilicity parameters [14–16]. Log k'_w is obtained from the extrapolated intercept of the plot of log k' versus the volume fraction of the organic modifier (φ). The extrapolation is based, among others, on Snyder's linear solvent strength (LSS) model, which assumes a linear relationship between log k' and φ over a limited range of binary mobile phase compositions; i.e., Eq. (2) below [17–19]:

$$\log k' = \log k'_w - S\varphi \tag{2}$$

where S is the slope of Eq. (2). S is solute-dependent solvent strength parameter.

The correlation between log k' or log k'_w and log P can be evaluated using the linear solvation energy relationship (LSER) of Abraham [20]. LSER provides the tools to assess the existing sim-

* Corresponding author. Tel.: +972 26585311; fax: +972 26586201.
E-mail address: eliga@chem.ch.huji.ac.il (E. Grushka).

ilarity between the chromatographic retention process and the n-octanol/water partitioning system since the model sheds light on the intermolecular interactions that contribute to the partition processes in both systems [20–25]. In its general form, the LSER model can be written as Eq. (3):

$$\log k' = c + eE + sS + aA + bB + vV \tag{3}$$

The model is constructed of a sum of products. Each product represents an intermolecular interaction. E, S, A, B and V are solute descriptors, each representing a specific property of the solute: the excess molar refraction E, dipolarity/polarizability S, effective hydrogen bond acidity A, effective hydrogen bond basicity B and the Mc Gowan's characteristic volume V. The lower case constants e, s, a, b and v are system constants that are complementary to the solute descriptors. The e coefficient is a measure of the difference in the ability of the mobile and the solvated stationary phase to interact with the solute lone pair electrons, s relates to the ability to participate to dipole–dipole and dipole–induced dipole interactions, a and b refer, respectively, to the ability to receive a proton and donate a proton in an hydrogen bond formation, v pertains to the ability of the solute to create a cavity in the mobile and stationary phases and c is a fitting constant [26–28]. The system constants are determined by using multiple linear regression analysis for a set of experimentally obtained $\log k'$ values of neutral solutes with known solute descriptors. Each system constant, for a given system, provides a quantitative and qualitative interpretation of the contribution of that intermolecular interaction to the retention process. It is important to realize that each system constant represents the difference in that particular property between the mobile phase and the solvated stationary phase in the case of chromatography, or the difference between the water and the n-octanol phases in the extraction system.

The lipophilicity assessment by reversed-phase HPLC evolved with the modern technologies of preparing new stationary phases. Recently, Kaliszan published a detailed review [29] on QSRRs where he reports on several studies on older and newer stationary phases for lipophilicity estimation.

In this study, we focus on a new generation of a polar embedded stationary phase, Supelco's Ascentis RP-Amide. The presence of free acidic silanol groups on the underlying silica of bonded phases has been identified as a drawback in the lipophilicity determination of ionized compounds [30,31]. One way to minimize the silanol effect is by the introduction of a polar amide group on the bonded alkyl chain in the vicinity of the binding site. The polar amide moieties can interact with the residual silica silanol through electrostatic and/or hydrogen-bonding interactions. The presence of the amide embedded group close to the silica surface greatly minimizes silanophilic interactions of the analytes with these groups [32,33].

Polar embedded stationary phases have been used successfully for mimicking the n-octanol/water partition process [34–40]. To improve the correlations between $\log P$ and retention parameters, inclusion of n-octanol in the chromatographic system is sometimes used to increase the similarity between the n-octanol/water partitioning system and the retention process. The addition of n-octanol either in the aqueous buffer or/and in the organic modifier seems to act as "a weak masking agent" again diminishing silanophilic interactions [41–43].

The Ascentis RP-Amide is based on high purity type B silica gel and is end capped. Although the Ascentis RP-Amide column should be an ideal column for lipophilicity measurements, no previous work was carried out to examine the suitability of this column for such measurements. The present work describes the ability of the Ascentis RP-Amide column to be a substitute for the n-octanol/water partitioning system for lipophilic determinations. The paper also investigates the influence of n-octanol in the mobile

phase on the correlations between $\log k'$ and $\log P$. LSER analysis is used to gain a better insight on the interactions of the column with the solutes and to provide a more meaningful comparison with the n-octanol/water partitioning system.

2. Experimental

2.1. Instrumentation

The column studied was an Ascentis RP-Amide column (50 mm × 4.6 mm I.D., 5 μm, Supelco, Bellefonte, USA).

The chromatographic system consisted of a LC-10AT VP pump (Shimadzu, Kyoto, Japan) and a UV-detector model SPD-10A (Shimadzu, Kyoto, Japan) operated at 254 nm. The mobile phase flow rate was 1.0 mL/min. The data were collected with a Hitachi D-2000 integrator (Hitachi, Ltd., Tokyo, Japan). During the study, the column temperature was maintained at 40 °C using a model TEP-1 temperature controller (Freed Electric, Haifa, Israel) and a water bath.

2.2. Chemicals

The water used throughout was purified and deionized with Seradest SD 2000 system (Seral, Ransbach, Germany). HPLC-grade methanol, used as the organic modifier, was purchased from J.T. Baker (Mallinckrodt Baker, Inc., Phillipsburg, NJ, USA). n-Octanol, approx. 99%, was purchased from Sigma (Sigma–Aldrich, Rehovot, Israel). For the n-octanol enriched mobile phase, the water was saturated with n-octanol to prepare the buffer and 0.25% (v/v) n-octanol was added to methanol.

$Na_2HPO_4 \cdot 7H_2O$ was purchased from Merck (Darmstadt, Germany). Buffers for the mobile phase at pH 3.0 and 7.0 were prepared by adjusting the pH of a 0.02 M disodium hydrogenphosphate salt solution with phosphoric acid (J.T. Baker, Mallinckrodt Baker, Deventer, The Netherlands). The mobile phase was prepared by mixing the appropriate buffer at a desired pH with methanol, in percentages ranging from 40% to 55%.

The solutes set can be classified into two groups: (a) neutral test solutes and (b) basic (local anesthetics, beta-adrenoceptor antagonists or β-blockers), acidic (NSAIDs: non-steroidal anti-inflammatory drugs) and neutral (steroid hormones) drugs. The neutral test solutes were of analytical reagent grade and were obtained from several sources. The steroid hormones (hydrocortisone-21-acetate, cortisone-21-acetate, prednisolone, and prednisone), the local anesthetics (lidocaine, procaine hydrochloride, prilocaine hydrochloride, mepivacaine hydrochloride, bupivacaine hydrochloride, tetracaine hydrochloride and dibucaine hydrochloride), the β-blockers (atenolol, alprenolol hydrochloride, metoprolol tartrate, nadolol, DL-propranolol hydrochloride, acebutolol hydrochloride, pindolol and sotalol hydrochloride) and the NSAIDs (ibuprofen, indoprofen, flurbiprofen, fenbufen and fenoprofen calcium salt hydrate) were purchased from Sigma (Sigma–Aldrich, Rehovot, Israel). Cortisone, corticosterone, hydrocortisone and naproxen were obtained from Fluka (Sigma–Aldrich, Rehovot, Israel). Solute solutions were prepared by dissolving the compounds in the mobile phase. The solutions were filtered through a 0.22 μm filter before injection.

For the acidic drugs (NSAIDs), the pH of the buffer in the mobile phase was kept at 3.0 to ensure that the solutes are in their neutral form.

$NaNO_3$, dissolved in the mobile phase, was injected as the void volume marker. The logarithm of the retention factor, $\log k'$, was used to express the retention data. The retention factor was corrected for the extra-column time (t_{ec}) according to: $(t_R - t_o)/(t_o - t_{ec})$ [44]. All measurements were performed in trip-

Table 1
Lipophilicity and physicochemical parameters of all solutes studied, in their neutral form, with and without *n*-octanol in the mobile phase.

n	Solute	Log P	With *n*-octanol in the mobile phase			Without *n*-octanol in the mobile phase		
			Log $k'_{w(o)}$	Slope	r^2	log $k'_{w(o)}$	Slope	r^2
1	Acetone	−0.24	−0.255 (±0.04)	0.365 (±0.08)	0.919	0.010 (±0.05)	0.872 (±0.11)	0.972
2	2-Butanone	0.29	0.301 (±0.04)	0.918 (±0.04)	0.995	0.767 (±0.10)	1.911 (±0.20)	0.979
3	3-Pentanone	0.99	0.898 (±0.03)	1.536 (±0.06)	0.997	1.222 (±0.02)	2.215 (±0.04)	0.999
4	2-Hexanone	1.38	1.499 (±0.04)	2.188 (±0.08)	0.997	1.869 (±0.03)	2.977 (±0.06)	0.999
5	2-Heptanone	1.98	2.156 (±0.04)	2.910 (±0.04)	0.999	2.540 (±0.07)	3.725 (±0.14)	0.997
6	2-Octanone	2.37	2.846 (±0.05)	3.703 (±0.10)	0.999	3.247 (±0.05)	4.556 (±0.10)	0.999
7	Acetophenone	1.58	1.585 (±0.02)	2.344 (±0.05)	0.999	1.921 (±0.04)	3.049 (±0.08)	0.999
8	Propiophenone	2.19	2.171 (±0.03)	2.908 (±0.06)	0.999	2.504 (±0.05)	3.622 (±0.10)	0.999
9	Butyrophenone	2.77	2.710 (±0.03)	3.448 (±0.06)	0.999	3.073 (±0.05)	4.220 (±0.11)	0.999
10	Valerophenone	3.15	3.298 (±0.03)	4.035 (±0.07)	0.999	3.693 (±0.06)	4.870 (±0.14)	0.998
11	Phenol	1.46	1.458 (±0.02)	2.183 (±0.03)	0.999	1.657 (±0.02)	2.639 (±0.04)	1.000
12	Hydroquinone	0.59	0.293 (±0.04)	1.234 (±0.08)	0.992	0.591 (±0.03)	1.801 (±0.06)	0.998
13	Resorcinol	0.8	0.617 (±0.04)	1.550 (±0.07)	0.995	0.939 (±0.03)	2.192 (±0.06)	0.999
14	Catechol	0.88	0.831 (±0.02)	1.638 (±0.03)	0.999	1.102 (±0.03)	2.202 (±0.06)	0.999
15	m-Aminophenol	0.21	0.103 (±0.04)	0.998 (±0.08)	0.988	0.397 (±0.02)	1.557 (±0.05)	0.998
16	o-Aminophenol	0.62	0.555 (±0.03)	1.369 (±0.05)	0.997	0.934 (±0.09)	2.167 (±0.18)	0.987
17	m-Nitrophenol	2	2.182 (±0.06)	3.053 (±0.12)	0.997	2.399 (±0.02)	3.532 (±0.05)	1.000
18	p-Cresol	1.94	1.945 (±0.02)	2.685 (±0.03)	1.000	2.159 (±0.03)	3.171 (±0.05)	0.999
19	m-Cresol	1.9	1.933 (±0.01)	2.670 (±0.04)	1.000	2.152 (±0.02)	3.168 (±0.05)	1.000
20	o-Cresol	1.95	1.981 (±0.02)	2.660 (±0.03)	1.000	2.187 (±0.02)	3.132 (±0.05)	0.999
21	Toluene	2.73	2.795 (±0.06)	3.301 (±0.12)	0.997	2.789 (±0.01)	3.410 (±0.01)	1.000
22	Ethylbenzene	3.15	3.310 (±0.02)	3.792 (±0.05)	1.000	3.355 (±0.02)	3.999 (±0.04)	1.000
23	Nitrobenzene	1.85	1.889 (±0.02)	2.494 (±0.08)	0.999	2.101 (±0.01)	2.986 (±0.06)	1.000
24	Benzonitrile	1.56	1.568 (±0.02)	2.351 (±0.04)	0.998	1.864 (±0.03)	2.996 (±0.04)	0.999
25	Chlorobenzene	2.84	2.941 (±0.05)	3.526 (±0.10)	0.998	2.936 (±0.01)	3.633 (±0.02)	1.000
26	Anisole	2.11	2.155 (±0.05)	2.734 (±0.10)	0.997	2.282 (±0.02)	3.091 (±0.04)	1.000
27	Caffeine	−0.07	0.070 (±0.04)	0.806 (±0.09)	0.977	0.768 (±0.04)	2.086 (±0.13)	0.992
28	Antipyrine	0.38	0.474 (±0.03)	1.348 (±0.07)	0.995	1.079 (±0.04)	2.471 (±0.08)	0.998
29	4-Chlorophenol	2.39	2.549 (±0.06)	3.301 (±0.12)	0.997	2.666 (±0.02)	3.625 (±0.04)	1.000
30	Aniline	0.9	0.859 (±0.03)	1.540 (±0.07)	0.996	1.120 (±0.01)	2.122 (±0.02)	1.000
31	3-Chloroaniline	1.88	1.926 (±0.05)	2.675 (±0.11)	0.996	2.120 (±0.02)	3.143 (±0.05)	1.000
32	Acetaminophen	0.51	0.245 (±0.03)	1.175 (±0.07)	0.994	0.721 (±0.02)	2.075 (±0.04)	0.999
33	m-Toluidine	1.4	1.375 (±0.02)	2.105 (±0.04)	0.999	1.645 (±0.02)	2.691 (±0.05)	0.999
34	o-Toluidine	1.32	1.310 (±0.02)	1.996 (±0.04)	0.999	1.579 (±0.02)	2.579 (±0.04)	1.000
35	o-Nitroaniline	1.85	1.829 (±0.05)	2.506 (±0.11)	0.997	2.081 (±0.02)	3.070 (±0.05)	0.999
36	p-Nitroaniline	1.39	1.425 (±0.05)	2.199 (±0.11)	0.995	1.763 (±0.03)	2.909 (±0.06)	0.999
37	m-Nitroaniline	1.37	1.409 (±0.05)	2.104 (±0.10)	0.996	1.725 (±0.03)	2.782 (±0.04)	0.999
38	Quinoline	2.03	1.767 (±0.02)	2.643 (±0.05)	0.999	2.063 (±0.04)	3.284 (±0.08)	0.999
39	3-Bromoquinoline	3.03	2.815 (±0.04)	3.552 (±0.08)	0.999	3.039 (±0.06)	4.080 (±0.12)	0.998
40	2-Naphtol	2.7	2.805 (±0.06)	3.615 (±0.12)	0.998	2.994 (±0.04)	4.063 (±0.09)	0.999
41	Naphthalene	3.37	3.463 (±0.06)	4.061 (±0.12)	0.998	3.491 (±0.02)	4.231 (±0.05)	1.000
Steroid hormones								
42	Cortisone-21-acetate	2.1	2.573 (±0.07)	3.575 (±0.14)	0.997	3.853 (±0.13)	5.962 (±0.26)	0.996
43	Corticosterone	1.94	2.352 (±0.06)	3.254 (±0.12)	0.997	3.435 (±0.10)	5.281 (±0.21)	0.997
44	Cortisone	1.47	1.820 (±0.06)	2.834 (±0.12)	0.997	2.904 (±0.11)	4.853 (±0.22)	0.996
45	Hydrocortisone	1.61	1.987 (±0.06)	2.924 (±0.12)	0.997	3.021 (±0.09)	4.853 (±0.19)	0.997
46	Prednisolone	1.62	2.030 (±0.06)	3.024 (±0.13)	0.997	3.048 (±0.10)	4.924 (±0.21)	0.997
47	Prednisone	1.46	1.776 (±0.05)	2.820 (±0.11)	0.997	2.811 (±0.10)	4.754 (±0.21)	0.996
48	Hydrocortisone-21-acetate	2.19	2.648 (±0.07)	3.605 (±0.14)	0.997	3.790 (±0.14)	5.750 (±0.23)	0.997
NSAIDs-pH 3.0								
49	Naproxen	3.18	3.095 (±0.02)	4.120 (±0.05)	1.000	3.731 (±0.12)	5.107 (±0.25)	0.995
50	Flurbiprofen	4.16	3.996 (±0.02)	5.059 (±0.04)	1.000	4.621 (±0.13)	6.015 (±0.27)	0.996
51	Indoprofen	2.77	2.483 (±0.02)	3.620 (±0.05)	1.000	3.414 (±0.12)	5.101 (±0.26)	0.995
52	Fenbufen	3.2	3.221 (±0.02)	4.319 (±0.04)	1.000	3.953 (±0.09)	5.465 (±019)	0.998
53	Fenoprofen Ca^{2+} salt hydrate	3.9	3.664 (±0.02)	4.701 (±0.03)	1.000	4.334 (±0.12)	5.740 (±0.25)	0.996
54	Ibuprofen	3.97	4.052 (±0.02)	4.955 (±0.03)	1.000	4.661 (±0.11)	5.884 (±0.23)	0.997

licate and the log k' values reported are the average of three replicates. Retention factors were obtained at various concentrations of MeOH in the mobile phase (40%, 45%, 50%, and 55%) and were extrapolated to 100% water, to obtain log k'_w, using a conventional least square procedure. In our study, the notation of log $k'_{w(o)}$ represents log k'_w obtained using mobile phase containing *n*-octanol. Three β-blockers (atenolol, nadolol and sotalol hydrochloride) were removed from the set of the basic drugs due to the poor statistics associated with the extrapolation to log k'_w. For these three compounds, the linear solvent strength model was not applicable. Their retention factors increase with the increasing of the methanol in the mobile phase. We noticed that these

compounds differ from the others by their very low lipophilicity values. However, we did include the three drugs in the set of the basic drugs for the results at 40% MeOH. The notation of log k'_{40} refers to the logarithm of the retention factor at 40% MeOH without *n*-octanol in the mobile phase. The notation of log $k'_{40(o)}$ will be used when *n*-octanol is added to the mobile phase.

2.3. Lipophilicity and structural parameters

Experimental *n*-octanol/water partition coefficients, log P, were obtained from Syracuse Research Corporation's PhysProp database and from the shake-flask data of Lombardo [35]. Log D values for
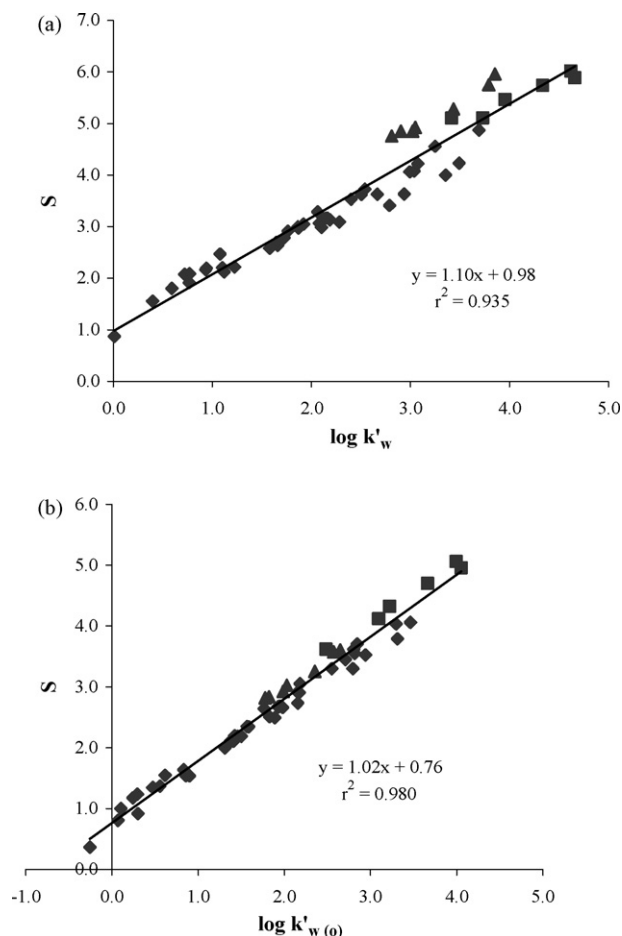
Fig. 1. (a) Relationship between S and $\log k'_w$ for the whole set of 54 neutral compounds without n-octanol in the mobile phase: (♦) neutral solutes; (■) NSAIDs; (▲) steroid hormones. (b) Relationship between S and $\log k'_{w(o)}$ for the whole set of 54 neutral compounds in the presence of n-octanol in the mobile phase (for symbols see (a)).

monoprotic bases were calculated from the corresponding $\log P$ values using the following expression as suggested by Scherrer and Howard [45] (Eq. (4)):

$$\log D^{pH} = \log P_{oct} + \log \frac{1}{1 + 10^{pK_a - pH}} \qquad (4)$$

For monoprotic acids, the exponent $pK_a - pH$ in the right hand side of Eq. (4) is replaced by $pH - pK_a$. For the calculations of $\log D^{7.0}$, the pK_a (calculated) of the drugs were obtained through the American Chemical Society's SciFinder Scholar program.

The solute descriptors for Abraham's linear solvation energy relationship model were obtained from the literature and reported in a previous article [46].

Multiple linear regression analysis and the related statistical functions were performed using Microsoft's Excel.

3. Results and discussions

3.1. Application of the linear solvent strength theory model

The logarithm of the extrapolated retention factor, $\log k'_w$, is often used to estimate the lipophilic character of the solutes. Usually, $\log k'_w$ is obtained by extrapolating $\log k'$ values measured with an organic modifier in the mobile phase to neat aqueous mobile phase. In the present work, we measured k' values using mobile phases containing from 40% to 55% methanol. Two sets of mea-

Table 2
Correlations between $\log P$ and $\log k'_w$ at pH 7.0 and 3.0, according to Eq. (1): $\log P = a + b \log k'_w$ with and without n-octanol in the mobile phase for the neutral solutes and neutral drugs.

Set of solutes (equation number)	pH	n	b (slope)	a (intercept)	r²	s	F
The whole set of solutes, without n-octanol (7)	7.0, 3.0	54	0.84 (±0.05)	−0.10 (±0.13)	0.845	0.407	284
The whole set of solutes, with n-octanol (8)	7.0, 3.0	54	0.96 (±0.02)	+0.03 (±0.05)	0.966	0.190	1496
Neutral solutes and NSAIDs in their neutral form, without n-octanol (9)	7.0, 3.0	47	0.94 (±0.03)	−0.21 (±0.06)	0.966	0.203	1287
Neutral solutes and NSAIDs in their neutral form, with n-octanol (10)	7.0, 3.0	47	0.97 (±0.02)	+0.05 (±0.04)	0.983	0.143	2634
Neutral solutes, without n-octanol (11)	7.0	41	1.00 (±0.03)	−0.29 (±0.07)	0.957	0.196	867
Neutral solutes, with n-octanol (12)	7.0	41	0.93 (±0.02)	+0.09 (±0.04)	0.982	0.125	2189
Steroid hormones, without n-octanol (13)	7.0	7	0.70 (±0.05)	−0.51 (±0.07)	0.974	0.054	185
Steroid hormones, with n-octanol (14)	7.0	7	0.85 (±0.02)	−0.07 (±0.05)	0.996	0.021	1252
NSAIDs, without n-octanol (15)	3.0	6	1.07 (±0.12)	−0.89 (±0.50)	0.951	0.137	78
NSAIDs, with n-octanol (16)	3.0	6	0.89 (±0.11)	+0.47 (±0.37)	0.945	0.145	69

surements were done. In the first set, the mobile phase contained buffer (at pH 7.0 and at pH 3.0) and methanol only. The data from this set of measurements were used to obtain $\log k'_w$ through linear regression of Eq. (2). In the second set of measurements, *n*-octanol was added to the mobile phase as well. The data from this set of runs was used to calculate $\log k'_{w(o)}$, again by using Eq. (2). In addition to $\log k'_w$ (or $\log k'_{w(o)}$) values we also obtain the slopes, *S*, values of the regression lines for the solutes in their neutral form. The results are given in Table 1 where, for the sake of completion, $\log P$ values are given as well.

The slope *S* of the correlation equation between $\log k'$ and φ (Eq. (2)) represents a significant structural parameter, which is related to the specific hydrophobic surface area [43,47]. The slope *S* is considered to be depended on the size of the solute and on the retention mechanism of the solute in a given stationary/mobile phases system [18,19]. A linear relationship between *S* and $\log k'_w$ for a set of solutes indicates a similarity in the intermolecular interactions between these solutes and the stationary phase in particular when the methanol is the organic modifier used [48]. To investigate the effect of *n*-octanol in the mobile phase, we have compared the correlation between *S* and $\log k'_w$ for a set of 54 diverse neutral compounds in the two mobile phases. Fig. 1a (without *n*-octanol) and Fig. 1b (with *n*-octanol) show the plots of *S* versus $\log k'_w$ and $\log k'_{w(o)}$ for the complete set of the neutral solutes studied here. Two different mobile phases were used, with different pHs (7.0 and 3.0) of the aqueous component, to ensure the solute neutrality. In spite of the diversity of the investigated compounds, Fig. 1 shows good linear correlations between *S* and $\log k'_w$ or $\log k'_{w(o)}$. Eqs. (5) and (6) give the regression equations, and the regression statistics, for Fig. 1a and b, respectively.

$$S = 1.10(\pm 0.04) \log k'_w + 0.98(\pm 0.10)$$
$$n = 54; \quad r^2 = 0.935; \quad s = 0.331; \quad F = 746 \tag{5}$$

$$S = 1.02(\pm 0.02) \log k'_{w(o)} + 0.76(\pm 0.04)$$
$$n = 54; \quad r^2 = 0.980; \quad s = 0.160; \quad F = 2515 \tag{6}$$

The values in parentheses represent 95% confidence limits; *n* is the number of the compounds; r^2 represents the squared correlation coefficient; *s* is the standard error and *F* gives the Fischer's test value. The good linear correlations in Fig. 1a and b are directly related to the solute neutrality and similarity of the interactions between these solutes and the Ascentis RP-Amide stationary phase. The addition of *n*-octanol to the mobile phase has a significant effect on the S–$\log k'_w$ correlation. The slope of the line is closer to unity while the intercept was significantly lower. The presence of *n*-octanol in the mobile phase improves the correlation between *S* and $\log k'_{w(o)}$ as can be seen from the statistics of the regression line in Eq. (6). The *n*-octanol in the mobile phase plays an important role in ensuring a consistent retention mechanism of the solutes in the chromatographic system. Our results confirm the findings of Giaginis et al. on the influence of the *n*-octanol as a mobile phase additive on the S–$\log k'_w$ linearity in a BDS C18 column [47]. However, in our case, a good correlation between *S* and $\log k'_w$ was obtained even without *n*-octanol in the mobile phase. Our results are contrary to the findings of Liu et al. who showed a poor correlation in a Discovery RP-Amide C16 column [42].

3.2. Correlation between $\log P$ or $\log D^{7.0}$ and $\log k'_w$

In this study, we explore the ability of the Ascentis RP-Amide column to mimic the partitioning mechanism of the *n*-octanol/water system. We examine the effect of the addition of *n*-octanol to the mobile phase on the correlation between the $\log P$ values of a large set of solutes and their extrapolated retention fac-
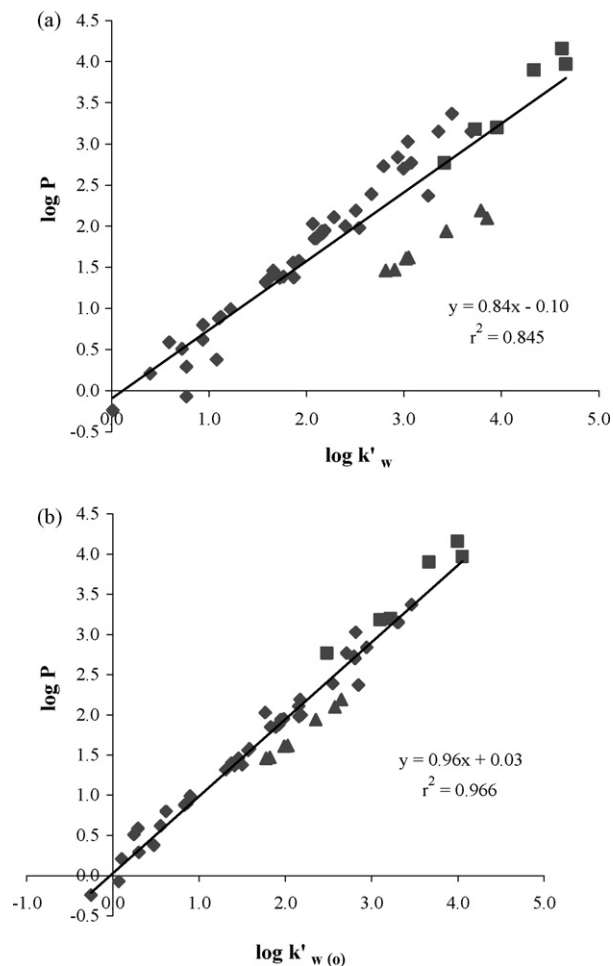

Fig. 2. (a) Relationship between $\log P$ values and $\log k'_w$ for the whole set of 54 solutes without *n*-octanol in the mobile phase (for symbols see Fig. 1a). (b) Relationship between $\log P$ and $\log k'_{w(o)}$ for the whole set of 54 neutral solutes in presence of *n*-octanol in the mobile phase (for symbols see Fig. 1a).

tors $\log k'_{w(o)}$. The solutes set included neutral solutes, acidic and neutral drugs. Thus, to ensure that all the solutes were in their neutral state, the pH of the mobile phase was adjusted accordingly (see Section 2). The correlated data are shown in Fig. 2. Eqs. (7) and (8) summarize the statistics of the regression lines without and with *n*-octanol in the mobile phase, respectively:

$$\log P = 0.84(\pm 0.05) \log k'_w - 0.10(\pm 0.13)$$
$$n = 54; \quad r^2 = 0.845; \quad s = 0.407; \quad F = 284 \tag{7}$$

$$\log P = 0.96(\pm 0.02) \log k'_{w(o)} + 0.03(\pm 0.05)$$
$$n = 54; \quad r^2 = 0.966; \quad s = 0.190; \quad F = 1496 \tag{8}$$

From Fig. 2 and Eqs. (7) and (8), it is clear that the addition of *n*-octanol to the mobile phase substantially improves the correlation between $\log P$ and $\log k'_w$; the slope of the regression line is closer to unity and the intercept is smaller and almost zero. According to Minick [41], a slope of unity is indicative of the homoenergetic nature of the two partitioning processes; i.e. that the free energy changes in both processes are equivalent. The effect of *n*-octanol on the correlations between $\log P$ and $\log k'_w$ or $\log k'_{w(o)}$ is summarized in Table 2 where the data is grouped according to the different families of the solutes. In general, the addition of *n*-octanol to the mobile phase improved the correlation of the fit (r^2 increases) and the slope of the correlation approaches unity. The most noticeable change in r^2 and in the slope of the line is for the steroid hormones (neutral

Table 3
Lipophilicity and physicochemical parameters of the ionized drugs obtained at pH 7.0, with and without n-octanol in the mobile phase.

n	Drugs	Log $D^{7.0}$	With n-octanol in the mobile phase		Without n-octanol in the mobile phase	
			Log $k'_{w(o)}$	r^2	Log k'_w	r^2
β-Blockers						
1	Alprenolol hydrochloride	0.94	1.849	0.998	2.554	0.995
2	Acebutolol hydrochloride	−0.39	1.070	0.991	1.591	0.992
3	Pindolol	−0.45	0.754	0.975	1.080	0.990
4	DL-Propranolol hydrochloride	1.34	1.993	0.999	2.527	0.995
5	Metoprolol tartrate	−0.48	0.919	0.987	1.448	0.989
Local anesthetics						
6	Lidocaine	0.90	2.452	0.984	3.251	0.997
7	Procaine hydrochloride	−0.10	0.815	0.929	1.426	0.998
8	Prilocaine hydrochloride	1.11	1.939	0.985	2.529	0.995
9	Mepivacaine hydrochloride	0.83	1.912	0.988	2.530	0.997
10	Dibucaine hydrochloride	2.44	3.458	0.982	4.201	0.992
11	Bupivacaine hydrochloride	1.98	3.350	0.989	4.063	0.996
12	Tetracaine hydrochloride	2.25	2.893	0.988	3.721	0.996
NSAIDs						
13	Naproxen	0.58	1.944	0.994	2.534	0.999
14	Flurbiprofen	1.30	2.796	0.997	3.278	0.999
15	Indoprofen	0.16	1.701	0.994	2.503	1.000
16	Fenbufen	0.75	2.626	0.997	2.985	0.999
17	Fenoprofen Ca^{2+} salt hydrate	1.10	2.345	0.993	2.956	0.999
18	Ibuprofen	1.38	2.610	0.994	3.093	0.999

drugs), examined at pH 7.0. It should be noted that the steroid hormones (symbolized by ▲), in the absence of n-octanol, Fig. 2a, form a separated group off the correlation line. However, in Fig. 2b, with n-octanol in the mobile phase, the steroid hormones shift closer to the correlation line. As in the case of the correlation between S and log k'_w (Fig. 1), the addition of n-octanol to the mobile phase leads to a better correlation between log P and log $k'_{w(o)}$ for the complete set of solutes. In light of the above discussion, we have removed the steroid hormones from the complete set of solutes and investigated the correlation between log P and log k'_w for the neutral solutes and the NSAIDs only (see Table 2, Eqs. (9) and (10)). In both cases, with and without n-octanol, highly significant correlations are obtained for the complete set of solutes. However, the addition of n-octanol improves the correlation. To continue, we analyzed the effect of n-octanol on the correlation between log P and log k'_w for the neutral solutes only without any of the drug molecules (see Table 2, Eqs. (11) and (12)). The addition of n-octanol to the mobile phase improves the linear relationship between log P values of neutral solutes and log $k'_{w(o)}$. The improvement is evident from the statistics of the fits: the squared correlation coefficient, r^2, is higher in presence of n-octanol and the standard error, s, is smaller. For the NSAIDs as a set by itself, the correlation did not change considerably by the addition of n-octanol to the mobile phase (see Table 2, Eqs. (15) and (16)). Finally, the presence of n-octanol in the mobile phase enhances the similarity between the partitioning system of n-octanol/water and the Ascentis RP-Amide stationary phase making them more homoenergetic [41].

The polar embedded reversed-phase Ascentis RP-Amide column shows improved linearity of the correlation between log P and log k'_w or log $k'_{w(o)}$. The polar amide group added to the long alkyl chain modified the interactions of the classical octadecylsilane stationary phase leading to stronger interactions with the hydrogen bonds donor groups of polar compounds [49]. Therefore, the presence of the polar amide group in the stationary phase allows the chromatographic system to better emulate the interactions that occur in the extraction system.

For the ionized drugs, the correlation was established between the distribution coefficients log D and log k'_w or log $k'_{w(o)}$, both obtained at pH 7.0 for both basic and acidic drugs. The log $D^{7.0}$, log k'_w and log $k'_{w(o)}$ values are given in Table 3. The range of methanol content used for the correlations was the same as was used in the previous part of the study. The ionized drugs include the basic β-blockers, local anesthetics and the acidic NSAIDs. As mentioned in the experimental part, three β-blockers were removed from the basic drugs due to the absence of linearity between log k' and φ, the volume fraction of methanol. These solutes, which have very low lipophilicity values, differ from the other drugs of the same family. Their retention times barely changed with increasing methanol in the mobile phase. Therefore, they were removed from the set of the basic drugs. Table 4 summarizes the correlations data for the ionized drugs.

In Table 4, in addition to correlating all the ionized solutes as one group, we also examined individually the acidic group and the basic group. For the three groups, in the presence of n-octanol, the slopes of the correlations are almost unity. The intercepts of the correlation lines are substantially different from zero and are negative. In the presence of n-octanol, the intercepts are less negative. The squared correlation coefficients, r^2, are smaller than those obtained

Table 4
Correlations between log $D^{7.0}$ and log k'_w at pH 7.0 according to Eq. (1): ($\log D^{7.0} = a + b \log k'^{7.0}_w$) with and without n-octanol in the mobile phase for the ionized drugs.

Drugs (equation number)	n	b (slope)	a (intercept)	r^2	s	F
Ionized drugs, without n-octanol (17)	18	0.92 (±0.09)	−1.61 (±0.26)	0.868	0.328	105
Ionized drugs, with n-octanol (18)	18	0.99 (±0.10)	−1.19 (±0.23)	0.854	0.345	94
Acidic drugs, without n-octanol (19)	6	1.35 (±0.34)	−3.04 (±0.98)	0.802	0.234	16
Acidic drugs, with n-octanol (20)	6	0.94 (±0.28)	−1.31 (±0.66)	0.741	0.267	11
Basic drugs, without n-octanol (21)	12	0.94 (±0.09)	−1.55 (±0.26)	0.907	0.334	98
Basic drugs, with n-octanol (22)	12	1.05 (±0.10)	−1.18 (±0.22)	0.913	0.323	105

for the neutral compounds as are the other statistics of the regression. For the NSAIDS, the addition of n-octanol to the mobile phase does not greatly affect the correlation line as compared to the case where the drugs were in their neutral form (see Table 4, Eqs. (19) and (20)).

Reasonable linear correlations between the calculated $\log D^{7.0}$ and $\log k'_{w(o)}$ were obtained for the basic drugs, with a little improvement in presence of n-octanol in the mobile phase (see Table 4, Eq. (22)). In general, the lower correlation coefficients of the regression lines for the ionized solutes are not surprising in light of their wide diversity. At pH 7.0, the β-blockers and the local anesthetics are positively charged and almost fully protonated. At the same pH, residual silanols in conventional silica based chromatographic column are ionized and negatively charged. Therefore, it is difficult to obtain a good correlation between $\log D^{7.0}$ and $\log k'_w / \log k'$ due to the strong ion-exchange interactions [39]. These electrostatic interactions can be reduced by the addition of n-decylamine to the mobile phase as described previously by Lombardo et al. [35]. In our case, we have not added n-decylamine to the mobile phase just for keeping on the same experimental conditions of other studied columns that we want to compare.

3.3. Correlation between $\log P$ and isocratic $\log k'_{40}$

We investigated the use of an isocratic retention factor, $\log k'_\%$ (% represents the percent methanol in the mobile phase), as the chromatographic parameter for the correlation with $\log P$ values. While the correlation between $\log k'_w$ and $\log P$ is more valid, the determination of $\log k'_w$ by extrapolation can be, at time, problematic due to (a) the relatively narrow range of the organic modifier used for the extrapolation, (b) possible dependence on the nature of the organic modifier and (c) length of time of the measurements. Therefore, we wish to examine and see if a quick estimate of the lipophilicity can be obtained from a single chromatographic measurement using a binary mobile phase. In the present work, four methanol compositions from 40% to 55% were used, with and without n-octanol in the mobile phase. For each one of these compositions, the correlation between $\log P$ and $\log k'_\%$ was examined. The results showed that the mobile phase with the lowest (40%) methanol concentration yielded the best correlations. For example, Fig. 3 shows the correlation between $\log P$ and $\log k'_{40}$, with and without n-octanol in the mobile phase. The solutes included the complete set of 54 compounds discussed above (neutral solutes, acidic and neutral drugs). Eqs. (23) and (24) summarize the statistics of the regression lines:

$$\log P = 1.53(\pm 0.05)\ \log k'_{40} + 0.44(\pm 0.06)$$
$$n = 54;\ r^2 = 0.940;\ s = 0.253;\ F = 816 \tag{23}$$

$$\log P = 1.61(\pm 0.05)\ \log k'_{40(o)} + 0.54(\pm 0.05)$$
$$n = 54;\ r^2 = 0.960;\ s = 0.210;\ F = 1206 \tag{24}$$

In all cases, the slope of the correlation line is greater than unity and the intercept is positive. Interestingly, the steroid hormones appear again as a separated group in the absence of n-octanol in the mobile phase but a better r^2 value is obtained as compared to the correlation between $\log P$ and $\log k'_w$ (Fig. 2a).
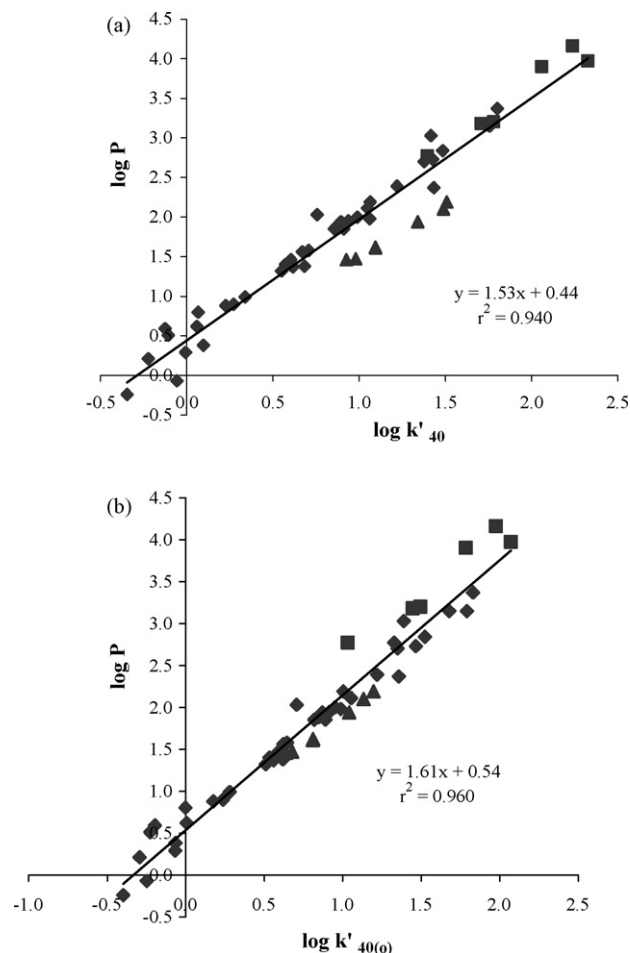

Fig. 3. (a) Relationship between $\log P$ values and $\log k'_{40}$ for the whole set of 54 solutes without n-octanol in the mobile phase (for symbols see Fig. 1a). (b) Relationship between $\log P$ and $\log k'_{40(o)}$ for the whole set of 54 neutral solutes in presence of n-octanol in the mobile phase (for symbols see Fig. 1a).

In view of the above results, the correlation of $\log k'_{40}$ or $\log k'_{40(o)}$ with $\log D^{7.0}$ should be examined. Table 5 summarizes the correlations of the various ionized drugs and the relevant statistics. The three β-blockers that were removed previously due to extrapolation difficulties are included both in the complete ionized drugs set and in the basic drugs set.

Satisfactory correlations are obtained between the lipophilic parameters and the isocratic retention factors at 40% methanol in the mobile phase. For the acidic drugs set (see Table 5, Eqs. (27) and (28)) and the basic drugs set (see Table 5, Eqs. (29) and (30)), the regression coefficients of the linear correlations between $\log D^{7.0}$ with $\log k'_{40}$ and $\log k'_{40(o)}$ were better than the values obtained for the extrapolated $\log k'_w$ and $\log k'_{w(o)}$. However, the correlation, at 40% methanol, of the whole set of ionized drugs has a lower regression coefficient (see Table 5, Eqs. (25) and (26)). These results agree with the observations of Pagliara et al. [34] who characterized a

Table 5
Correlations between $\log D^{7.0}$ and $\log k'_{40}$ at pH 7.0 according to Eq. (1): ($\log D^{7.0} = a + b\log k'$) with and without n-octanol in the mobile phase for the ionized drugs.

Drugs (equation number)	n	b (slope)	a (intercept)	r^2	s	F
Ionized drugs, without n-octanol (25)	21	1.81 (±0.19)	−1.13 (±0.21)	0.821	0.543	87
Ionized drugs, with n-octanol (26)	21	1.87 (±0.19)	−0.87 (±0.18)	0.835	0.521	96
Acidic drugs, without n-octanol (27)	6	1.68 (±0.25)	−0.34 (±0.19)	0.918	0.150	45
Acidic drugs, with n-octanol (28)	6	1.44 (±0.25)	+0.03 (±0.16)	0.893	0.172	33
Basic drugs, without n-octanol (29)	15	1.96 (±0.12)	−1.56 (±0.14)	0.957	0.309	290
Basic drugs, with n-octanol (30)	15	2.02 (±0.11)	−1.26 (±0.12)	0.960	0.297	314

Table 6
Comparison of the system constants for the extrapolated retention factors on the Gemini C18 and the Ascentis RP-Amide columns with and without *n*-octanol in the mobile phase and for the *n*-octanol/water system.

Separation system (equation number)		System constants						Statistics			
		c	v	e	s	a	b	r^2	SE	F	n
Gemini C18 column											
log $k'_{w\text{-without }n\text{-octanol}}$	(31)	−0.23	4.19	0.27	−0.84	−0.53	−2.84	0.99	0.12	484	41
log $k'_{w(o)\text{-with }n\text{-octanol}}$	(32)	−0.16	3.75	0.42	−1.09	−0.32	−2.98	0.98	0.13	421	41
Ascentis RP-Amide column											
log $k'_{w\text{-without }n\text{-octanol}}$	(33)	−0.17	4.24	0.21	−0.74	−0.14	−3.12	0.984	0.12	442	41
log $k'_{w(o)\text{-with }n\text{-octanol}}$	(34)	−0.14	4.15	0.39	−0.94	−0.14	−3.50	0.983	0.14	400	41
n-octanol/water log *P* [21]		0.09	3.81	0.56	−1.05	0.03	−3.46	0.995	0.12	23162	613

Supelcosil LC-ABZ column, an older generation of an embedded amide column of Supelco, using a mobile phase with 40% methanol. Based on our results, we can recommend that isocratic retention factors obtained with mobile phases containing 40% methanol, with or without *n*-octanol in the mobile phase, can be used as a rapid method to obtain a quick estimation of the lipophilic character of the solutes.

3.4. Application of the linear solvation energy relationship model for neutral compounds

To extend and complement the present study on the ability of the Ascentis RP-Amide column to emulate the *n*-octanol/water partitioning system, the solvation parameter model [20] was applied. Through LSER, a comparison of the intermolecular interactions of non-ionized compounds in the chromatographic system and in the *n*-octanol/water partitioning system can be made. The model was applied here to the logarithm of the extrapolated retention factor without (log k'_w) and with (log $k'_{w(o)}$) *n*-octanol in the mobile phase. From the solutes' log k'_w values, the system constants for the Ascentis RP-Amide chromatographic system were calculated using multiple linear regression of Eq. (3). In general, the larger the magnitude of the system constant, the greater the importance of that interaction to the retention mechanism in chromatography or to the partitioning process in the extraction system. Additionally, in the chromatographic system, a positive sign of the coefficient means that the interaction favors the stationary phase thus contributing to longer retention times while a negative sign indicates more favorable interactions with the mobile phase and consequently, shorter retention times. In the *n*-octanol/water system, a positive system constant is indicative of stronger interactions with the *n*-octanol phase thus contributing to a larger log *P* value whereas a negative system constant points out to stronger interactions with the aqueous phase, resulting in lower log *P* values. Hence, the LSER model affords a quick comparison of the intermolecular interactions contributing to the retention in Ascentis RP-Amide chromatographic system and in the *n*-octanol/water extraction system.

Table 6 shows the chromatographic system constants for the extrapolated log $k'_{w(o)}$ as well as for log k'_w. For the sake of comparison, the table includes data for the Ascentis RP-Amide column investigated in this study (see Table 6, Eqs. (31) and (32)) and for the Gemini C18 column that was studied by us previously (see Table 6, Eqs. (33) and (34)) [50]. The Gemini C18 column was examined using identical experimental conditions and with the same set of neutral solutes [50]. Also, Table 6 includes the system constants based on shake-flask log *P* values from Abraham [21]. For both columns, the addition of *n*-octanol to the mobile phase changes each one of the system constants of the extrapolated retention factors toward a better resemblance to the *n*-octanol/water partitioning system.

The Tanaka's radar plot [51] in Fig. 4 is used to compare the calculated system constants from measured log $k'_{w(o)}$ on the Ascentis RP-Amide system with available constants of the *n*-octanol/water extraction system [21]. In the radar plot, each axis represents a different system constant. The great advantage of the radar plot is that it provides a rapid visual comparison of the constants of the two systems. The absolute values of the system constants are scaled to fit the radar plot scale, which is between 0 at the origin and 5 at the highest point. From Fig. 4, the similarity between the Ascentis RP-Amide column, when *n*-octanol is in the mobile phase (log $k'_{w(o)}$), and the *n*-octanol/water partitioning system (log *P*) is obvious. The intermolecular interactions between the solutes and the Ascentis RP-Amide plus *n*-octanol in the mobile phase and the *n*-octanol/water partitioning system are very similar. The similarity between the two systems explains the very good correlation between log *P* and log $k'_{w(o)}$ as seen in Fig. 2b.

Fig. 4 and Table 6 show that the two main factors influencing the retention and partitioning in the two processes are the *b* and *v* constants. The large negative values of *b* are due to the strong acidic hydrogen-bonding capabilities of the aqueous mobile phase in the chromatographic system and of the water phase in the extraction system as compared with the stationary phase and the *n*-octanol phase of the two systems. The more negative *b* value in the *n*-octanol/water partitioning system is due, most likely, to the presence of solvated *n*-octanol in the water phase. Of the two columns shown in Table 6, the *b* constant of the Ascentis RP-Amide column, with *n*-octanol in the mobile phase, is the closest, in fact almost identical, to the *b* constant of the *n*-octanol/water partitioning system. The great similarity between these two systems is assumed to be due to the presence of the embedded amide group
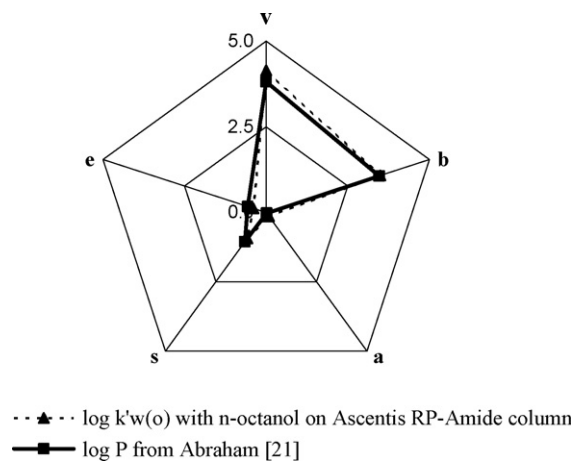

Fig. 4. Radar plot of the system constants for the Ascentis RP-Amide column with *n*-octanol (log $k'_{w(o)}$) and for the *n*-octanol/water extraction system (log *P* from the Abraham LSER model [21]).

on the alkyl chains of the Ascentis RP-Amide bonded phase, which blocks interactions with the residual silanols. The amide group is not present in the Gemini C18 column and therefore its *b* constant is not as close to that of the extraction system.

The second important constant *v* measures the relative ability of the solute to create a cavity in the solvated stationary phase and in the mobile phase. The positive sign of *v* is due to unfavorable cavity formation in the aqueous mobile phase, which means stronger interactions with the stationary phase. In the *n*-octanol/water partitioning system, the *n*-octanol phase, being able to form hydrogen bonds, is more cohesive than the Ascentis RP-Amide phase or than the Gemini C18 phase and thus the *v* coefficient is smaller in that system. However, the larger *v* constant of the Ascentis RP-Amide column, both with and without *n*-octanol in the mobile phase, points out the more hydrophobic character of this phase in comparison with the Gemini C18 column and the *n*-octanol/water partitioning system. For both columns, the *n*-octanol enriched mobile phase leads to a greater similarity to the *n*-octanol/water partitioning system.

The increase in system constant *e* with the addition of *n*-octanol points out to stronger polarizable interactions of the solutes with the two stationary phases than with the mobile phase and in similarity to the interactions with the *n*-octanol phase in the partitioning system. The negative *s* coefficient means that the chromatographic mobile phase with *n*-octanol is more dipolar than the stationary phases, similar to the water in the partitioning system. The small negative *a* coefficient indicates that the mobile phase is a little hydrogen-bond acceptor than the two stationary phases. The lower *a* constant value in the case of the Ascentis RP-Amide stationary phase, is due to the embedded amide group in the bonded stationary phase since it reduces the difference in hydrogen-bond basicity of the mobile and stationary phases. In the *n*-octanol/water partitioning system, the two phases are also almost identical in their hydrogen-bond basicity. Thus, the similarity between the *a* constants of the Ascentis RP-Amide system and the *n*-octanol/water partitioning system. The addition of *n*-octanol to the mobile phase in the Ascentis RP-Amide column does not affect the difference in the basicity of the two phases as is the case of the Gemini C18 column.

The above discussion can be summarized as follows. Based on the system constants *v*, *e*, and *s*, calculated using $\log k'_{w(o)}$, the Gemini C18 column behaves closer to the *n*-octanol/water system. On an other hand, from the *a* and *b* system constants the Ascentis RP-Amide column is more similar to the partitioning system. The solvation parameter model provides the understanding of the intermolecular forces responsible for the retention of neutral solutes on the two reversed-phase chromatographic systems and for the partitioning of these solutes in the *n*-octanol/water extraction system. The results of the above analysis afford a better choice of the suitable column for an accurate lipophilicity determination by chromatography.

4. Conclusions

The Ascentis RP-Amide column can mimic well the *n*-octanol/water partition process by suitable choice of chromatographic conditions and thus be used for lipophilicity determination. Extrapolated retention factors, $\log k'_w$ of a set of diverse compounds have been determined using four methanol contents in two mobile phases: one without *n*-octanol and the second with *n*-octanol. The addition of *n*-octanol enhanced the relationship between the slope (*S*) of the extrapolation lines and the extrapolated $\log k'_w$. Better correlations between $\log P$ values and the extrapolated $\log k'_{w(o)}$ were obtained for the enriched *n*-octanol mobile phase measurements. In addition, very good correlations were obtained between $\log P$

values and the logarithms of the isocratic retention factors at 40% methanol, $\log k'_{40}$. The Ascentis RP-Amide column seems to be a convenient tool for lipophilicity assessment for a wide range of solutes.

The Abraham's solvation parameter model (LSER) was employed to study the intermolecular interactions that contribute to the retention process in the Ascentis RP-Amide phase. Tanaka's radar plot was used for an easy visual comparison of the two systems showing the great similarity between them. The LSER system constants for the Ascentis RP-Amide column were found to be very similar to the LSER constants of the *n*-octanol/water extraction system. In particular, there is a close similarity in the hydrogen-bond related constants *a* and *b*. The LSER analysis corroborated the good correlations between $\log P$ and $\log k'_w$ found in the present work.

Also, the solvation parameter model was applied to compare two stationary phases investigated using identical experimental conditions: the Ascentis RP-Amide and the Gemini C18 previously studied by us [50]. The LSER investigation shows that there are some differences and some similarities between the two columns but, in general, the two columns behave closely to each other and both can be used for rapid lipophilicity determination.

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