European Journal of Medicinal Chemistry 47 (2012) 202–205

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

The effect of ionized species on microsomal binding

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ARTICLE INFO

Article history:
Received 2 September 2011
Received in revised form
21 October 2011
Accepted 21 October 2011
Available online 4 November 2011

Keywords:
Microsomal binding
Linear free energy relationship
Protonated base cations
Carboxylate anions
Microsomal binding of ions

ABSTRACT

The effect of neutral molecules on microsomal binding is known through studies by Austin and co-workers, but the effect of ionised species has hitherto not been elucidated. The present work sets out to determine the role of ionised species on microsomal binding. Data on microsomal binding obtained by Austin and co-workers have been analyzed by the method of Abraham and Acree that includes descriptors for neutral molecules, protonated base cations and carboxylate anions. An LFER has been obtained that includes neutral molecules, cations and anions in the same equation. It is shown that carboxylic acid anions bind to microsomes about 18 times less than the corresponding neutral carboxylic acids, but that protonated bases bind as strongly as the corresponding neutral bases. We interpret the stronger binding than expected of protonated bases as due to interaction with the phosphate groups on the phospholipids in the microsomes. Comparison with partition into a cerasome membrane suggests that this interaction corresponds to about a ten to twenty-fold increase in binding to microsomes.

1. Introduction

The rate of metabolic drug oxidation is an important facet of predictions of in vivo metabolic clearance of compounds in humans. Measurements of the rate of microsomal degradation of drugs are routinely used to assess metabolic drug oxidation. However, the use of such data in predicting human in vivo metabolic clearance can be confounded by the non-specific binding of compounds to the microsomes within the in vitro incubation. This binding will reduce the observed rate of degradation, and this effect needs to be accounted for during the process of scaling the data from in vitro to an in vivo prediction [1]. The measurement of microsomal binding is far less amenable to high throughput determination than is the measurement of rate of degradation. Therefore, the development of an understanding of the molecular and structural properties that control the binding, and of in silico methods for predicting the extent of binding, can be of great value as a surrogate for experimental measurements [2].

The extent of microsomal binding is usually reported as a free fraction (*fu*), defined by Eq. (1)

$$fu = [n(\mathrm{aq})/\{(n(\mathrm{aq}) + n(\mathrm{mic}))\}]^*[\{V(\mathrm{aq}) + V(\mathrm{mic})\}/V(\mathrm{aq})] \quad (1)$$

where n(aq) and n(mic) are the amounts of a drug in water and the microsomes, and V(aq) and V(mic) are the volumes of water and the microsomes. The quantity $(1 - fu)/fu$ has been shown to be proportional to an equilibrium constant [3], so that log $[(1 - fu)/fu]$ is then a free energy quantity and can be examined through linear free energy relationships (LFERs). However, it is important to recognise that an observed free fraction is dependent on the concentration of microsomal protein used in the experimental procedure. Consequently the analysis of *fu* data should, where possible, utilize data generated using a constant microsomal protein concentration. If this is not possible for all data, then Eq. (2) can be used to transform an *fu* value (fu_1) observed at microsomal protein concentration C_1 to an *fu* value (fu_2) at the desired microsomal protein concentration C_2 [3].

$$fu_2 = 1/[(C_2/C_1)^*(1 - fu_1)/fu_1 + 1] \quad (2)$$

A number of studies into the prediction of the extent of microsomal binding from calculated or measured molecular properties have been described [2–5]. These all highlight the influence of lipophilicity and charge type (acid/base/neutral) as important determinants of microsomal binding, but little else is known about other factors that control the binding process. Austin et al. [3] carried out measurements of binding of drugs to rat liver microsomes and used their data, together with data of Obach [6,7] on human liver microsomes and some data scaling using Eq. (2), to develop a model for the microsomal binding. For neutral drugs, it

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doi:10.1016/j.ejmech.2011.10.043

Table 1
Values of $SP = \log [(1 - fu)/fu]$, the fraction of cations and anions present at pH 7.4, and descriptors for the indicated species.

Species	*SP*	*F*(BH^+)	*F*(Anion)	*E*	*S*	*A*	*B*	*V*	J^+	J^-
Albendazole	−0.10	0.00	0.00	1.97	1.96	0.65	1.08	1.9475	0.000	0.000
Alprazolam	−0.66	0.00	0.00	2.90	2.50	0.00	1.55	2.2041	0.000	0.000
Amiodarone		0.00	0.00	3.33	2.48	0.00	1.37	3.7536	0.000	0.000
Amiodarone, cation	2.70	0.95	0.00	3.18	4.95	2.78	0.00	3.7751	3.350	0.000
Astemizole		0.00	0.00	3.10	2.84	0.19	1.95	3.5564	0.000	0.000
Astemizole, cation	1.92	0.90	0.00	2.95	6.52	3.39	0.00	3.5779	3.488	0.000
Betaxolol		0.00	0.00	1.18	1.51	0.24	1.79	2.5745	0.000	0.000
Betaxolol, cation	−0.21	0.99	0.00	1.03	5.51	2.43	0.00	2.596	2.244	0.000
Carbamazepine	−0.83	0.00	0.00	2.15	1.90	0.50	1.15	1.8106	0.000	0.000
Cerivastatin		0.00	0.00	2.23	2.22	1.10	2.65	3.6218	0.000	0.000
Cerivastatin, anion	−0.27	0.00	0.99	2.38	6.73	0.00	6.06	3.6003	0.000	3.223
Clomipramine		0.00	0.00	1.79	1.39	0.00	1.10	2.5239	0.000	0.000
Clomipramine, cation	1.40	0.99	0.00	1.64	3.78	1.71	0.00	2.5454	2.367	0.000
Clozapine		0.00	0.00	2.56	1.52	0.25	1.70	2.431	0.000	0.000
Clozapine, cation	0.45	0.80	0.00	2.41	5.29	1.63	0.00	2.4525	3.713	0.000
Glyburide		0.00	0.00	2.74	3.85	0.85	2.01	3.5583	0.000	0.000
Glyburide, anion	−0.66	0.00	0.99	2.89	8.51	0.00	4.93	3.5368	0.000	3.019
Indomethacin		0.00	0.00	2.24	1.47	0.58	0.58	2.5299	0.000	0.000
Indomethacin, anion	−0.63	0.00	0.99	2.39	5.62	0.00	3.35	2.5084	0.000	2.990
Isradipine	0.29	0.00	0.00	2.09	1.65	0.13	1.65	2.7088	0.000	0.000
Mebendazole	−0.37	0.00	0.00	2.74	2.60	0.71	1.22	2.1257	0.000	0.000
Methocarbamol	−0.72	0.00	0.00	1.32	2.05	0.60	1.40	1.7712	0.000	0.000
Oxaprozin		0.00	0.00	1.87	2.39	0.61	1.14	2.0279	0.000	0.000
Oxaprozin, anion	−0.83	0.00	0.99	2.02	5.82	0.00	3.69	2.0064	0.000	2.536
Phensuximide	−0.48	0.00	0.00	1.36	1.80	0.00	1.02	1.4435	0.000	0.000
Pimozide		0.00	0.00	2.51	2.54	0.33	1.70	3.4743	0.000	0.000
Pimozide, cation	2.15	0.94	0.00	2.36	5.78	3.16	0.00	3.4958	2.850	0.000
Promethazine		0.00	0.00	2.05	1.32	0.00	1.11	2.2832	0.000	0.000
Promethazine, cation	0.91	0.98	0.00	1.90	3.77	1.51	0.00	2.3047	2.695	0.000
Propafenone		0.00	0.00	1.83	2.11	0.25	1.75	2.8252	0.000	0.000
Propafenone, cation	0.75	0.99	0.00	1.68	5.69	3.08	0.00	2.8467	2.378	0.000
Sulindac		0.00	0.00	2.28	3.09	0.59	1.28	2.5711	0.000	0.000
Sulindac, anion	−0.79	0.00	0.99	2.43	7.01	0.00	3.88	2.5496	0.000	2.699
Tamoxifen		0.00	0.00	2.02	2.00	0.00	1.28	3.1747	0.000	0.000
Tamoxifen, cation	2.52	0.95	0.00	1.87	4.51	2.53	0.00	3.1962	2.317	0.000
Thioridazine		0.00	0.00	2.70	2.70	0.00	1.15	2.9017	0.000	0.000
Thioridazine, cation	2.04	0.99	0.00	2.55	4.52	3.33	0.00	2.9232	2.296	0.000
Trimeprazine		0.00	0.00	2.05	1.68	0.00	1.09	2.4241	0.000	0.000
Trimeprazine, cation	1.05	0.98	0.00	1.90	3.89	2.04	0.00	2.4456	2.387	0.000
Trioxasalen	0.21	0.00	0.00	1.50	1.50	0.00	0.72	1.6735	0.000	0.000
Verapamil		0.00	0.00	1.81	3.17	0.00	2.37	3.7861	0.000	0.000
Verapamil, cation	0.23	0.97	0.00	1.66	7.70	4.33	0.00	3.8076	2.404	0.000
Propranolol		0.00	0.00	1.84	1.43	0.44	1.31	2.148	0.000	0.000
Propranolol, cation	0.10	0.99	0.00	1.69	4.31	2.07	0.00	2.1695	2.432	0.000
Imipramine		0.00	0.00	1.15	1.45	0.00	1.04	2.4015	0.000	0.000
Imipramine, cation	0.72	0.99	0.00	1.00	3.66	2.02	0.00	2.423	1.611	0.000
Chlorpromazine		0.00	0.00	2.16	1.57	0.00	1.01	2.4056	0.000	0.000
Chlorpromazine, cation	0.91	0.99	0.00	2.01	3.65	1.84	0.00	2.4271	2.494	0.000
Diphenhydramine		0.00	0.00	1.31	1.11	0.00	1.22	2.1872	0.000	0.000
Diphenhydramine, cation	−0.39	0.97	0.00	1.16	3.94	1.46	0.00	2.2087	2.244	0.000
Diltiazem		0.00	0.00	2.66	2.14	0.00	2.22	3.1365	0.000	0.000
Diltiazem, cation	−0.80	0.67	0.00	2.51	6.85	2.51	0.00	3.158	3.908	0.000
Desipramine		0.00	0.00	1.62	1.86	0.01	0.94	2.2606	0.000	0.000
Desipramine, cation	0.88	0.99	0.00	1.47	3.62	2.79	0.00	2.2821	1.453	0.000
Amitriptyline		0.00	0.00	1.92	1.37	0.00	1.08	2.3996	0.000	0.000
Amitriptyline, cation	1.05	0.99	0.00	1.77	3.72	1.63	0.00	2.4211	2.491	0.000
Quinidine		0.00	0.00	2.47	1.23	0.37	1.97	2.5512	0.000	0.000
Quinidine, cation	−0.37	0.94	0.00	2.32	5.81	1.23	0.00	2.5727	4.157	0.000
Prednisone	−0.10	0.00	0.00	2.14	3.58	0.36	1.89	2.7116	0.000	0.000
Diazepam	−0.29	0.00	0.00	2.17	1.78	0.00	1.27	2.0739	0.000	0.000
Methoxsalen	−0.89	0.00	0.00	1.61	1.60	0.00	0.80	1.4504	0.000	0.000
Triazolam	−0.55	0.00	0.00	2.64	2.20	0.00	1.65	2.3265	0.000	0.000

was shown that the water–octanol partition coefficient, as log *P*(oct), led to a useful LFER. Basic compounds that were protonated at pH 7.4 exhibited enhanced microsomal binding due to electrostatic interactions between the protonated base and the phosphate groups on the phospholipids in the microsomes [8,9]. This leads to binding of protonated bases being greater than expected, and, indeed, of the same order as binding of the neutral bases. Thus log $[(1 - fu)/fu]$ could be correlated against log *P*(oct) for both neutral and basic drugs. For acidic drugs that were ionized at pH 7.4, no such enhanced binding could take place and log $[(1 - fu)/fu]$ was then correlated against log *D*(oct), where *D*(oct) is the partition coefficient for acidic compounds at pH 7.4. The full LFER was then given by Eq. (3) where *P*/*D* corresponds to *P*(oct) for neutral and basic compounds and *D*(oct) for acidic compounds.

$$\begin{aligned} &\mathrm{Log}[(1 - fu)/fu] = 0.53 \log P/D - 1.42 \\ &N = 37, SD = 0.411, R^2 = 0.82, F = 160.0 \end{aligned} \quad (3)$$

In Eq. (3) and elsewhere, N is the number of data points, SD is the regression standard deviation, R is the correlation coefficient and F is the F-statistic. Eq. (3) is a perfectly reasonable equation for the prediction of further values of fu for drugs. However, it does not yield information as to the structural factors in drugs that lead to strong (or weak) microsomal binding, and in particular it does not provide quantitative information on the effect of ionization of acidic and basic drugs. The aim of the present work is to investigate these areas.

$$\begin{aligned}\mathrm{Log}[(1-fu)/fu] &= -1.221 - 0.763S + 0.437A - 0.444B + 1.452V + 0.283J^{+} + 1.215J^{-}\\ N &= 37, SD = 0.490, R^2 = 0.809, F = 21.1, \mathrm{PRESS} = 15.88, Q^2 = 0.579\end{aligned} \quad (6)$$

2. Methods

We use the same LFER, Eq. (4), that we have previously employed for numerous systems including partition from water to solvents [10], intestinal absorption [11], absorption into skin [12], blood–brain distribution [13] and permeation through the blood–brain barrier [14].

$$SP = c + e\cdot E + s\cdot S + a\cdot A + b\cdot B + v\cdot V \quad (4)$$

In Eq. (4) SP, the dependent variable, is a property of a series of solutes in a given system. In the present case SP will be log [(1 − fu)/fu]. The independent variables in Eq. (4) are descriptors of the neutral solutes, as we have discussed before [15,16]. E is the solute excess molar refractivity in units of (cm^3 mol^{-1})/10, S is the solute dipolarity/polarizability, A and B are the overall or summation hydrogen bond acidity and basicity, and V is the McGowan volume in units of (cm^3 mol^{-1})/100. The coefficients in Eq. (4) are not just fitting constants but reflect the chemistry of the system in question. In particular, the a-coefficient will reflect the hydrogen bond basicity of the system (because a hydrogen bond solute will interact with a system that is a hydrogen bond base) and the b-coefficient will reflect the hydrogen bond acidity of the system.

More recently, Abraham and Acree [17–20] have extended Eq. (4) to cover ions and ionic species such as protonated amine cations and carboxylate anions by inclusion of two extra terms $j^{+}\cdot J^{+}$ and $j^{-}\cdot J^{-}$ as in Eq. (5). Cations have $J^{-} = 0$, anions have $J^{+} = 0$ and neutral species have $J^{-} = J^{+} = 0$ so that Eq. (5) then reverts to Eq. (4). It is important to note that the descriptors E, S, A, B, and V, for ions are on exactly the same scales as descriptors for neutral molecules.

$$SP = c + e\cdot E + s\cdot S + a\cdot A + b\cdot B + v\cdot V + j^{+}\cdot J^{+} + j^{-}\cdot J^{-} \quad (5)$$

Eq. (5) has been applied to permeation through the blood–brain barrier [14] and to partition into cerasome membranes [21]. We aim to obtain information on microsomal binding of neutral drugs and ionic species through application of Eq. (5).

3. Results and discussion

The data we use are taken from Austin et al. [3] as values of log [(1 − fu)/fu] for neutral drugs, and drugs that were entirely (or almost entirely) present as either cations or anions under the experimental conditions, see Table 1. Austin et al. [3] limited their analysis to species for which $fu < 0.90$, and we have done the same. The descriptors for neutral species are in Table 1 and were either taken from previous work [10–14] or were obtained from literature data as set out before [14,16–21]. The descriptors for cations and anions were calculated using the equations listed previously [17], and are given in Table 1. Also given are the fractions of cations (BH^{+}) or anions present at pH 7.4. Application of Eq. (5) to the log [(1 − fu)/fu] values in Table 1 yielded the LFER, Eq. (6). PRESS and Q^2 are the leave-one-out cross validation statistics. The value of Q^2, the predicted R^2, is reasonable and suggests that there is no serious overfitting.

In Eq. (6) the term in $e\cdot E$ was not significant and was left out. A plot of calculated vs observed values of log [(1 − fu)/fu] is shown in Fig. 1. Points for neutral species, cations and anions are all randomly scattered around the line of best fit so that there is no specific deviation for one species as compared to another. Eq. (6) is not as good statistically as the simple Eq. (3) of Austin et al. [3], but it has the considerable advantage that values of log [(1 − fu)/fu] can be calculated for any species. Hence we are able to express quantitatively the ratios of cation binding to the corresponding neutral species, and the ratios of anion binding to the corresponding neutral species. For the 21 bases in Table 1, log [(1 − fu)/fu] for the base cations averages 0.13 ± 0.54 more than that for the neutral bases, which indicates that the base cations on average bind to microsomes about 1.3 times better than the neutral bases. However, from the SD of 0.54 log units we conclude that there is almost no difference in binding of a protonated base cation and the corresponding neutral base molecule. For the five acids in Table 1, log [(1 − fu)/fu] averages −1.26 less for the anions than the neutral acids, so that anions derived from carboxylic acids bind about 18 times less than the corresponding neutral acid molecule. Thus we now have a quantitative explanation as to why log P(oct) can be used for bases in Eq. (3) but why log D(oct) has to be used for carboxylic acids. In addition, it is now possible to use Eq. (3) to predict log[(1 − fu)/fu] not only for neutral molecules but for numerous ionic species for which we already have descriptors, or for which descriptors can be estimated through equations that we have set out previously [17].

It would be useful to compare these results with other systems in which neutral molecules and ions are involved. Of the various systems for which we have all the coefficients in Eq. (5), the nearest in terms of coefficients is partition into the artificial membrane cerasome [21]. Values of the difference in partition of ions as

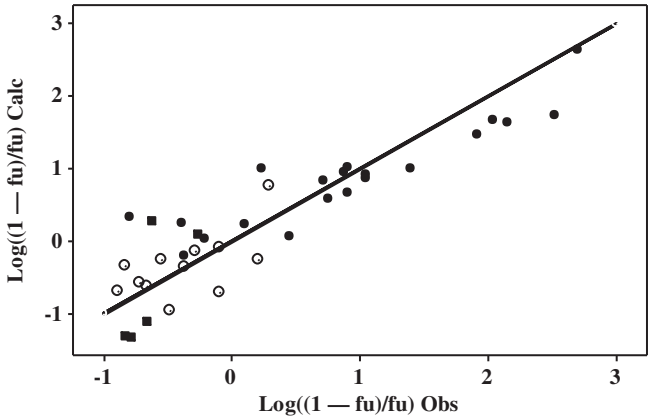

Fig. 1. A plot of calculated values of log [(1 − fu)/fu] on Eq. (4) against observed values: ○ neutral drugs, ● cations BH^{+}, ■ anions.

Table 2
Comparison of the effect of ionization on microsomal binding and partition into cerasome.

System	Log (anion) − Log (neutral)	Log (cation) − Log (neutral)
Microsomal binding	−1.26	+0.13
Cerasome partitioning	−0.60	−0.44

compared to corresponding neutral molecules are given in Table 2 for cerasome partition and microsomal binding. The electrostatic interactions between a protonated base and the phosphate groups in the microsomes [8,9] thus leads to an enhanced binding. By comparison to cerasome partitioning, we would expect that microsomal binding of protonated base cations would be less favoured than binding of the corresponding neutral molecules by about 0.66 log units, instead of a more favoured binding by about 0.57 log units. The enhanced binding thus amounts to 1.23 log units which is a 10–20 fold increase in binding.

Inspection of the coefficients in Eq. (6) shows that the main factor that influences microsomal binding of neutral drugs is the volume, or size, of the drug; the larger the volume the greater is the binding. The other terms are less important, but dipolarity ($s = -0.763$) and hydrogen bond basicity ($b = -0.444$) reduce binding and hydrogen bond acidity ($a = 0.437$) increases binding. The latter is commensurate with interactions between neutral molecules that are hydrogen bond acids and the phosphate groups on the phospholipids in the microsomes.

4. Conclusions

We have been able to set up an LFER for microsomal binding that includes neutral molecules, protonated base cations and carboxylate anions in the same equations. This enables, for the first time, a quantitative estimation to be made of the effect of ionization on microsomal binding, and provides a method for the prediction of microsomal binding of ionized species. We show that carboxylate anions bind about 18 times less than the corresponding neutral carboxylic acids, but that protonated bases bind to almost the same extent as the neutral bases. This is due to interaction between the protonated base and the phosphate groups in the microsome, an effect that is worth a factor of about 10–20 in binding to microsomes.

References

[1] R.S. Obach, The importance of nonspecific binding in in vitro matrices, its impact on enzyme kinetic studies of drug metabolism and implications for in vitro–in vivo correlations, Drug Metab. Dispos. 24 (1996) 1047–1049.
[2] H. Gao, S.J. Steyn, G. Chang, J. Lin, Assessment of in silico models for fraction of unbound drug in human liver microsomes, Expert Opin. Drug Metab. Toxicol. 6 (5) (2010) 533–542.
[3] R.P. Austin, P. Barton, S.L. Cockroft, M.C. Wenlock, R.J. Riley, The influence of nonspecific microsomal binding on apparent intrinsic clearance, and its prediction from physicochemical properties, Drug Metab. Dispos. 30 (2002) 1497–1503.
[4] D. Hallifax, J.B. Houston, Binding of drugs to hepatic microsomes: comment and assessment of current prediction methodology with recommendation for improvement, Drug Metab. Dispos. 34 (2006) 724–726.
[5] H. Gao, L. Yao, H.W. Mathieu, Y. Zhang, T.S. Maurer, M.D. Troutman, D.O. Scott, R.B. Ruggeri, J. Lin, In silico modeling of nonspecific binding to human liver microsomes, Drug Metab. Dispos. 36 (2008) 2130–2135.
[6] R.S. Obach, Nonspecific binding to microsomes: impact on scale-up of in vitro intrinsic clearance to hepatic clearance as assessed through examination of warfarin, imipramine and propranolol, Drug Metab. Dispos. 25 (1997) 1359–1369.
[7] R.S. Obach, Prediction of human clearance of twenty nine drugs from hepatic microsomal clearance data: an examination of in vitro half-life approach and nonspecific binding to microsomes, Drug Metab. Dispos. 27 (1999) 1350–1359.
[8] R.P. Austin, A.M. Davis, C.N. Manners, Partitioning of ionizing molecules between aqueous buffers and phospholipid vesicles, J. Pharm. Sci. 84 (1995) 1180–1183.
[9] S.D. Kramer, A. Braun, C. Jakits-Deiser, H. Wunderli-Allenspach, Towards the predictability of drug-lipid membrane interactions: the pH-dependent affinity of propranolol to phosphatidylinositol containing liposomes, Pharm. Res. 15 (1998) 739–744.
[10] M.H. Abraham, R.E. Smith, R. Luchtefeld, A.J. Boorem, R. Luo, W.E. Acree Jr., Prediction of solubility of drugs and other compounds in organic solvents, J. Pharm. Sci. 99 (2010) 1500–1515.
[11] Y.H. Zhao, J. Le, M.H. Abraham, A. Hersey, P.E. Eddershaw, C.N. Luscombe, D. Butina, G. Beck, B. Sherborne, I. Cooper, J.A. Platts, Evaluation of human intestinal absorption data and subsequent derivation of a quantitative structure-activity relationship (QSAR) with the Abraham descriptors, J. Pharm. Sci. 90 (2001) 749–784.
[12] M.H. Abraham, F. Martins, Human skin permeation and partition; general Linear Free-Energy Relationship analyses, J. Pharm. Sci. 93 (2004) 1508–1523.
[13] M.H. Abraham, A. Ibrahim, Y. Zhao, W.E. Acree Jr., A data base for partition of volatile organic compounds and drugs from blood/plasma/serum to brain, and an LFER analysis of the data, J. Pharm. Sci. 95 (2006) 2091–2100.
[14] M.H. Abraham, The permeation of neutral molecules, ions and ionic species through membranes: brain permeation as an example, J. Pharm. Sci. 100 (2011) 1690–1701.
[15] M.H. Abraham, Chem. Soc. Rev. 22 (1993) 73–83.
[16] M.H. Abraham, A. Ibrahim, A.M. Zissimos, J. Chromatogr. A 1037 (2004) 29–47.
[17] M.H. Abraham, W.E. Acree Jr., Equations for the transfer of neutral molecules and ionic species from water to organic phases, J. Org. Chem. 75 (2010) 1006–1015.
[18] M.H. Abraham, W.E. Acree Jr., Solute descriptors for phenoxide anions and their use to establish correlations of rates of reaction of anions with iodomethane, J. Org. Chem. 75 (2010) 3021–3026.
[19] M.H. Abraham, W.E. Acree Jr., The transfer of neutral molecules, ions and ionic species from water to ethylene glycol and to propylene carbonate; descriptors for pyridinium cations, New J. Chem. 34 (2010) 2298–2305.
[20] M.H. Abraham, W.E. Acree Jr., The transfer of neutral molecules, ions and ionic species from water to wet octanol, Phys. Chem. Chem. Phys. 12 (2010) 13182–13188.
[21] K. Zhang, M. Chen, G.K.E. Scriba, M.H. Abraham, A. Fahr, X. Lui, Linear free energy analysis of retention factors in cerasome electrokinetic chromatography intended for predicting skin permeation, J. Pharm. Sci. 100 (2011) 3105–3113.