Journal of Pharmaceutical and Biomedical Analysis 54 (2011) 893–899

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

journal homepage: www.elsevier.com/locate/jpba

Capillary electrochromatography as a new tool to assess drug affinity for membrane phospholipids

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ARTICLE INFO

Article history:
Received 21 September 2010
Received in revised form
19 November 2010
Accepted 23 November 2010
Available online 2 December 2010

Keywords:
IAM capillary electrochromatography
IAM-HPLC
Phospholipids
Biomembranes
Lipophilicity

ABSTRACT

This work proposes a new capillary electrochromatography (CEC) method for determination of drug partition in membrane phospholipids.

CEC experiments were carried out in a 100 μm (ID) fused-silica capillary, partially packed with a chromatographic phospholipid stationary phase, so-called Immobilized Artificial Membrane, IAM.PC.DD2. The observed retention values were corrected by both the electro-osmotic and electrophoretic mobility values, measured by capillary electrophoresis (CE) experiments, assuming the values of the logarithms of "chromatographic" affinity factors, $\log k_{CEC}$ as indexes of affinity for phospholipids. Analogously to biochromatography, all the values were determined with a totally aqueous mobile phase, or extrapolated to 100% aqueous buffer. The analytes were 16 structurally unrelated compounds, of basic, neutral, and acidic nature.

To evaluate the effectiveness of CEC data to describe partition in phospholipids, $\log k_{CEC}$ were related to both $\log P$ and $\log k_w^{IAM}$ values. $\log P$ are the lipophilicity values expressed as the logarithms of *n*-octanol/water partition coefficients and $\log k_w^{IAM}$ are the retention data measured by High Performance Liquid Chromatography (HPLC) on an IAM.PC.DD2 column, assumed as the reference values for phospholipid affinity.

Phospholipid affinity scale by CEC related to that achieved by HPLC, but only if two different subclasses were considered separately, i.e. protonated and unprotonated analytes; indeed, all the compounds protonated at the experimental pH value (7.0) were retained stronger in CEC than in HPLC.

This discrepancy may be due to the use of different buffers in CEC and HPLC since, to avoid the occurrence of a high current, the eluent in CEC experiments was of different composition and lower ionic strength than in HPLC.

CEC analyses were faster and required lower amounts of both solvent and stationary phase than HPLC; moreover, with the exception of only three analytes, all analyses were performed with 100% aqueous eluents avoiding time-consuming and tedious extrapolation procedures.

1. Introduction

Partition in biological membranes is the main step governing the passive passage of drugs through biological barriers and, consequently, the occurrence of several pharmacokinetic phenomena (e.g. intestinal absorption and blood–brain barrier passage).

It is usually related to lipophilicity, expressed as the logarithm of *n*-octanol/water partition coefficient, $\log P$ [1]. However, *n*-octanol is a neutral and isotropic phase, in contrast to the charged and anisotropic phospholipid bi-layer of biological membranes. Therefore $\log P$ is unable to take into account electrostatic intermolecular recognition forces [2,3] and may underestimate membrane interactions of basic compounds [4–8]. Therefore, since many years, phospholipids themselves have been proposed as an alternative to *n*-octanol in drug/membrane partition studies. In presence of water, phospholipids have a strong tendency to aggregate spontaneously to ordered, usually lamellar, bi-layer structures such as membranes or liposomes. Unfortunately, although partition in liposomes was demonstrated to yield interaction scales better mimicking membrane interactions than partition in *n*-octanol, the technique is not suitable for large scale application because its set up is very difficult and even more time-consuming and tedious than partition in *n*-octanol.

To date, the only adequate solution to these drawbacks is the HPLC (*H*igh *P*erformance *L*iquid *C*hromatography) retention measure on phospholipid stationary phases, the so-called Immobilized

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doi:10.1016/j.jpba.2010.11.037

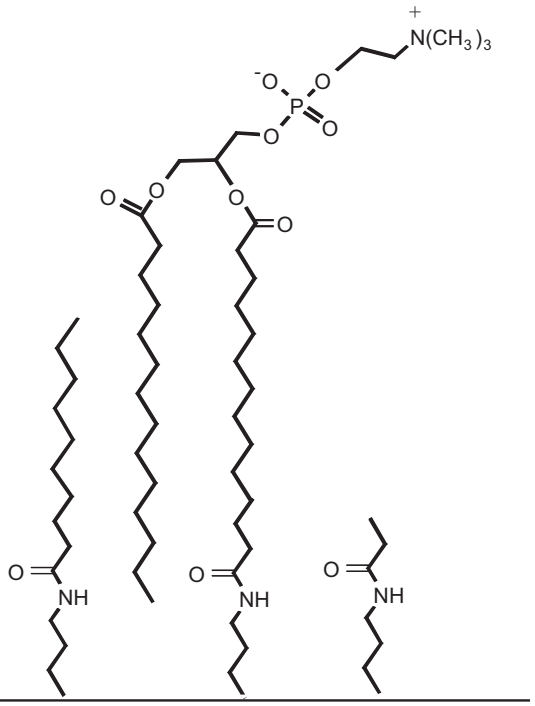

Fig. 1. Structure of IAM.PC.DD2 stationary phase. Silica-propylamine is linked to 1-myristoyl-2-[(13-carboxyl)-tridecanoyl]-*sn*-3-glycerophosphocoline (i.e. lecithin-COOH).

Artificial Membrane (IAM), such as IAM.PC.DD2 (Fig. 1). IAM-HPLC is a simple, reproducible, and fast method, combining the robustness of HPLC technique with a model of phospholipid partitioning.

Nevertheless, the possibility of measuring drug/phospholipid affinity by other methods than HPLC would be useful, mainly in industrial field, to perform faster and less expensive analyses.

For this purpose, some authors proposed either MEKC (*M*icellar *E*lectro*k*inetic *C*hromatography) or MEEKC (*M*icro *E*mulsion *E*lectro*k*inetic *C*hromatography) as alternative methods [9,10].

However, although both techniques demonstrated effective to measure partition in phospholipids, their reproducibility is negatively affected by the need of preparing micelles or liposomes as stationary phase.

The aim of the present work has been to investigate about the possibility of measuring drug partition between phospholipids and an aqueous phase by Capillary ElectroChromatography (CEC). It is a modern analytical technique used for qualitative and quantitative determination of both neutral and charged species and can be considered a hybrid technique that combines HPLC and capillary electrophoresis (CE) features, both as regards the separation process, involving multiple mechanisms, and as regards the advantages. CEC advantages include greater efficiency, a reduced consumption of organic solvents, small volumes of sample and reduced time of analysis as compared to HPLC method. In recent years the interest in this technique has significantly grown [11,12].

CEC measures were performed on a capillary packed with an IAM.PC.DD2 chromatographic stationary phase and the interaction component with phospholipids ($\log k_{CEC}$) was calculated by a suitable equation reported in literature [13]. Furthermore, the possible relationships between these data and both $\log P^N$ and $\log k_w^{IAM}$ were also investigated. $\log P^N$ are the *n*-octanol/water lipophilicity data of the neutral forms of analytes measured by the "shake-flask" method [1] and $\log k_w^{IAM}$ are the HPLC retention data measured on an IAM.PC.DD2 chromatographic column, i.e. a column packed with the same stationary phase used in CEC.

In the present study we considered 16 structurally unrelated drugs, i.e. atenolol, ranitidine, phenylpropanolamine, ephedrine, ketamine, tramadol, diphenhydramine, verapamil (bases ionized as cations at pH 7.0, the experimental pH value), theobromine, nicotinamide, caffeine, benzene, temazepam, oxazepam, toluene (unionized at the experimental pH value), and thiopental (an acidic molecule partly ionized as an anion at the experimental pH value) (Fig. 2).

The inclusion of ionized compounds in the set considered is important both to achieve an interaction scale with phospholipids non-collinear with the lipophilicity scale in *n*-octanol and to verify whether the unique behaviour of bases already observed in IAM-HPLC is also observed in CEC.

2. Materials and methods

2.1. Materials

All chemicals were obtained from commercial sources; they were of HPLC grade and used without further purification.

Acetonitrile, methanol (HPLC-grade) and trihydroxy methylaminomethane (Tris) were purchased from Carlo Erba (Milan, Italy). Potassium dihydrogen phosphate was from Honeywell Riedel-de Haën (Seelze, Germany). Sodium monohydrogen phosphate was from J.T. Baker (Deventer, The Netherlands). Ultrapure water was obtained using a Milli-Q water purification system from Millipore (Milford, MA, USA).

2.2. Liquid Chromatography

2.2.1. Instrumentation

A Shimadzu liquid chromatographic apparatus (LC-10AD), equipped with a 7725 Rheodyne injection valve (fitted with a 20 μL loop) and a SPD-10AV UV detector (Shimadzu), set at 254 nm wavelength, was used. Separations were carried out in a stainless steel column IAM-PC-DD2 (4.6 mm × 100 mm) (Regis Chemical Company, Morton Grove, IL, USA). The chromatograms were recorded by a 746 Data Module (Millipore).

2.2.2. Chromatographic conditions

The eluents were mixtures of 100 mM phosphate buffer at pH 7.0 (a mixture of sodium monohydrogen phosphate and potassium dihydrogen phosphate at a final concentration 100 mM) and different percentages of acetonitrile at a flow rate of 1.0 mL/min. The chromatography was carried out at room temperature. All samples were dissolved in methanol (ca. 10^{-3} M); 20 μL samples were injected in the chromatograph. Chromatographic retention data are expressed as the logarithm of the retention factor, $\log k$, defined as $\log k = \log[(t_r - t_0)/t_0]$, where t_r and t_0 are the retention times of the analyte and a non-retained compound (citric acid), respectively. $\log k$ values were determined with completely aqueous eluents for all the compounds eluting within 20 min (corresponding to $\log k \cong 1.0$). For solutes requiring the addition of an organic modifier in the eluent, $\log k$ values relative to 100% aqueous eluent were calculated by performing a polycratic method of extrapolation [14]: they were eluted with mobile phases containing acetonitrile in percentages (φ) ranging from 10 to 30% (v/v). Linear relationships between $\log k$ and φ values were found for all compounds in the range of eluent composition examined ($r^2 \geq 0.992$). The values referring to 100% aqueous eluent are indicated as $\log k_w^{IAM}$ values. All values of $\log k$ reported are the average of at least three measurements; the 95% confidence interval never exceeded 0.04 for each $\log k$ value. Possible occurrence of retention changes due to column ageing was monitored by checking the retention times of five test compounds (amlodipine, *p*-nitroaniline, toluene, isradipine, and ketoprofen). During the study no retention value of test

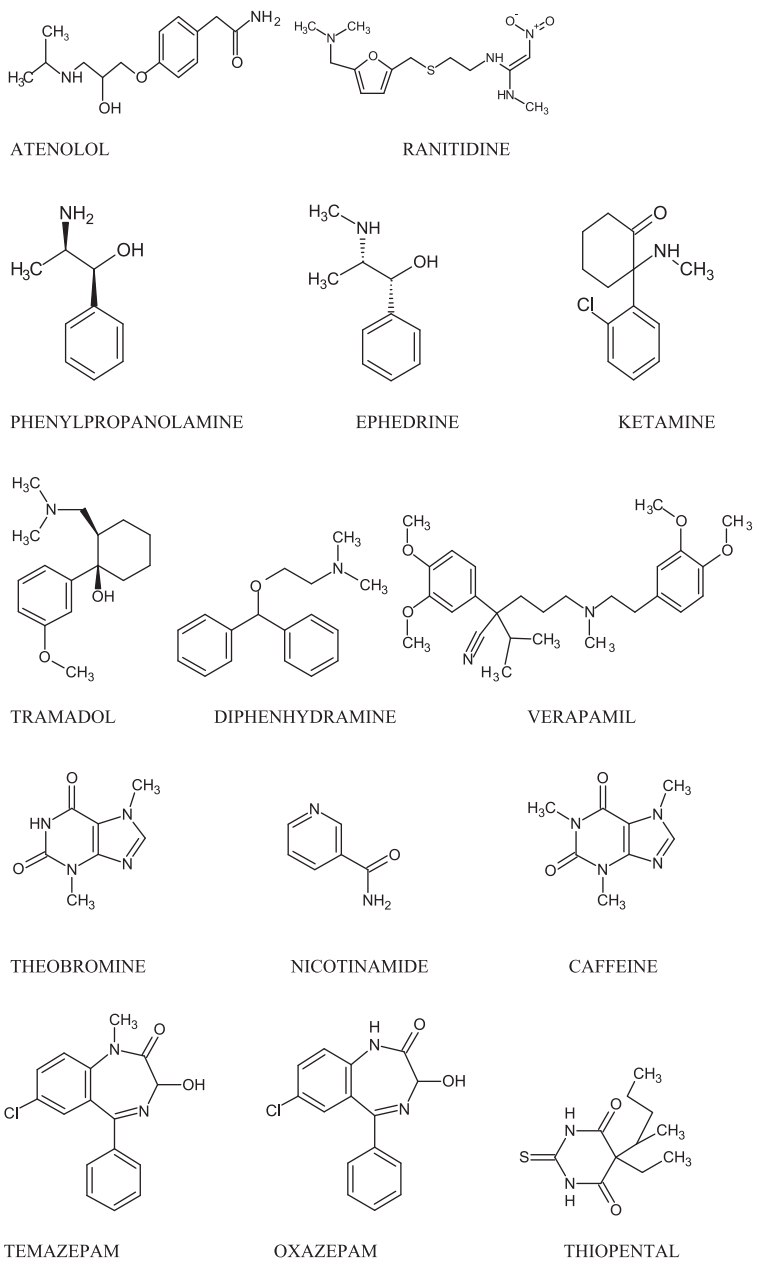

Fig. 2. Chemical structures of investigated compounds.

compounds changed more than 3% and no correction was done to the retention values experimentally determined for the analytes.

Partition coefficients in *n*-octanol of the neutral species, log P^N values, were taken from the literature [15,16] or calculated by the program ClogP for Windows Version 2.0 (Biobyte Corp. Claremont, CA, USA). pK_a values were calculated by the program ACD-pK_a DB-ACD labs version 7.0.

A commercially available statistical package for personal computer was used for linear regression analysis. Requirements of significant regression analysis were observed.

2.3. Capillary electrophoresis and capillary electrochromatography

2.3.1. Apparatus

An Agilent Capillary Electrophoresis System (Agilent Technologies, Waldbronn, Germany), equipped with a diode array UV detector and external nitrogen pressure was used for CEC experiments using a fused-silica capillary of 100 μm ID (375 μm OD) (Composite Metal Services, Hallow, UK) partially packed with phospholipid phase IAM.PC.DD2 (12 μm).

The capillary column was prepared in laboratory according to the procedure previously described with minor modifications [17]. One end of the capillary was connected to a mechanical temporary frit to retain the packing material, the other to a HPLC precolumn which was used as reservoir for the slurry and was connected to the LC pump. The slurry was prepared by adding few mg of stationary phase to 1 mL acetone.

IAM.PC.DD2 stationary phase was not suitable for frit fabrication, so Lichrospher 100 RP_{18}, 5 μm silica stationary phase (Merck, Darmstadt, Germany) was used in order to obtain frits with good mechanical stability.

Briefly the capillary was packed with the C_{18} silica particles for about 10 cm. Then the capillary was flushed with double-distilled water to remove the packing solvent from the column. The frits were prepared sintering the particles with a heating coil. The

temporary frit was removed and excess packing material was eliminated by pumping double-distilled water through the column. After that, the capillary was filled with the IAM.PC.DD2 stationary phase until the desired length and then again C_{18} SP was put inside the capillary to make the second (outlet) frit. The excess of the phase was removed by flushing with the mobile phase.

Finally, the capillary was cut at the desired length and the detection window was formed removing the polyimide capillary coating. The total length of the capillary was 33.0 cm and the effective length 8.4 cm. The outlet frit (7.0 cm from the inlet) was protected with a layer of epoxy resin.

During runs the capillary was pressurized at both ends applying 10 bar pressure. The analytes were detected at 254 nm. The experiments were carried out at 20 °C and the applied voltage was −10 kV. The reversed-polarity mode was used to perform the separation in the shorter part of the capillary; however analytes moved towards the cathode [18]. The analytes were injected by pressure using the short-end injection method applying −12 bar × 0.2 min followed by a plug of mobile phase at −12 bar × 0.1 min. The amount of each analyte injected can be roughly estimated as about 5 ng. The stock solution of mobile phase (100 mM) pH 7.0 was used to prepare the background electrolyte (BGE) mixing the appropriate volume of stock solution and acetonitrile to obtain a final concentration of 10 mM. The experiments were performed in a 10 mM trihydroxymethyl-aminomethane (Tris) buffer at pH 7.0, containing in some cases (diphenhydramine, tramadol, and verapamil) 20, 25, or 30% of acetonitrile.

The parameter we assumed to express the interaction between an analyte and the IAM.PC.DD2 stationary phase in capillary electrochromatography, indicated as the logarithm of "chromatographic" retention factor, $\log k_{CEC}$, was derived from CEC retention measures according to the following equation [13]:

$$k_{CEC} = \frac{t_m \cdot (1 + k_e) - t_0}{t_0} \quad (1)$$

where t_m and t_0 are the retention times of the analyte and a non-retained compound (methanol), respectively, measured in CEC; k_e is the velocity factor defined as: $k_e = \mu_p/\mu_o$; μ_p = electrophoretic mobility; μ_o = electro-osmotic mobility.

From a practical point of view, all compounds were previously analyzed in CE to measure the respective values of μ_p (electrophoretic mobility); it is easily obtained as the difference between μ_r (apparent mobility) and μ_o (electro-osmotic mobility): $\mu_p = \mu_r - \mu_o$.

The apparent mobility values for each analyte were calculated from the retention times observed in CE according to the following equation:

$$\mu_r = \frac{L_d \cdot L_{tot}}{V \cdot t_r} \quad (2)$$

where L_d is the capillary length between the inlet and the detection point, L_{tot} is the total length of the capillary, V is the potential difference applied (10,000 V), and t_r is the retention time expressed in seconds.

Electro-osmotic mobility (μ_o) of all compounds was assumed as equal to the apparent mobility (μ_r) of benzyl alcohol, calculated according to Eq. (2).

Since in CEC benzyl alcohol can partition in the stationary phase, t_0 values in CEC were measured as the retention time of methanol (the solvent used to prepare the analytical samples).

2.3.2. Sample preparation

In CEC analysis, stock solutions of analytes were prepared in methanol (1 mg/mL) and stored at 4 °C. Each solution was diluted in methanol at the final concentration of 0.1 mg/mL before injection.

Table 1
Lipophilicity, pK_a, and phospholipid retention data for the compounds considered.

Compound	log P	pK_a	logk_w^{IAM}	log k_{CEC}
Atenolol	0.16	9.16	0.765	0.509
Benzene	2.13	n.i.	0.720**	0.280
Caffeine	−0.07	0.73	0.680	−0.574
Diphenhydramine	3.27	9.10	2.170**	2.343**
Ephedrine	0.93	9.60	0.973**	0.996
Ketamine	2.18	6.46	1.339**	1.365
Nicotinamide	−0.37	3.54	−0.179	−1.133
Oxazepam	2.24	n.i.	2.163**	1.465
Phenylpropanolamine	0.67	9.40	0.579**	0.944
Ranitidine	0.27	8.40	0.812	1.154
Temazepam	2.19	n.i.	1.697**	1.353
Theobromine	−0.72	9.90*	−0.088	−0.929
Thiopental	2.85	7.60*	1.328**	0.525
Toluene	2.73	n.i.	1.210**	0.674
Tramadol	2.63	8.30	1.347**	1.789**
Verapamil	3.79	8.90	3.085**	2.604**

n.i. = non-ionizable; * acid compound; ** value obtained by extrapolation.

For CE experiments, the same stock solutions (1 mg/mL) were diluted with methanol at final concentrations in the range 10^{-4} to 10^{-5} mg/mL.

3. Results and discussion

The set of molecules studied includes basic, neutral, and acid compounds (thiopental and theobromine). Since lipophilicity and pK_a are physico-chemical properties of analytes fundamental in CE and CEC methods, the values, calculated as above mentioned, are summarized in Table 1 together with the logarithms of IAM-HPLC retention factors ($\log k_w^{IAM}$) and CEC "chromatographic" retention factors ($\log k_{CEC}$). At the experimental pH (7.0) only atenolol, diphenhydramine, ephedrine, ketamine, phenylpropanolamine, ranitidine, tramadol, and verapamil are ionized in an appreciable extent (as cations); thiopental is partly ionized as an anion, whereas the other compounds are in their neutral forms due to either their non-ionizable nature (benzene and toluene) or too weak pK_a values (theobromine, oxazepam, temazepam, nicotinamide, and caffeine).

The determination of retention factors on IAM column ($\log k_w^{IAM}$) was performed by HPLC with eluent at pH 7.0 to obtain experimental conditions as close as possible to the physiological pH and compatible with the stability of the stationary phase. Moreover, buffer concentration was 100 mM being this value demonstrated as the one effective to produce phospholipid affinity scales related to the interactions with biological membrane [2,3,5,6,19]. The compounds with $\log P^N$ values ≤ 0.6 eluted in a reasonable time (i.e. within 30 min) with a completely aqueous mobile phase. All other compounds had to be eluted with mobile phases containing various acetonitrile fractions and the relative $\log k_w^{IAM}$ values were calculated by performing a polycratic method of extrapolation [14].

Fig. 3 reports the plot of $\log k_w^{IAM}$ versus $\log P^N$ for the compounds considered, in comparison to that for 39 neutral compounds.

Actually, in our previous work [19] we found that $\log k_w^{IAM}$ values of 39 structurally unrelated neutral compounds with $\log P > 0.5$ relate by a single relationship with the respective $\log P^N$ values. The relationship is expressed by the following equation:

$$\underset{n=39}{\log k_w^{IAM}} = \underset{r^2=0.954}{0.939(\pm 0.034)\ \log P^N} - \underset{s=0.236}{0.897(\pm 0.089)} \quad (3)$$

In this and the following equations, n denotes the number of molecules considered in the derivation of the regression equation, r is the correlation coefficient, and s is the standard error of the estimate. Numbers in parentheses represent the standard error of the regression coefficients.

As can be seen in Fig. 3, only six of the considered compounds (i.e. benzene, diphenhydramine, ketamine, tramadol, toluene, and

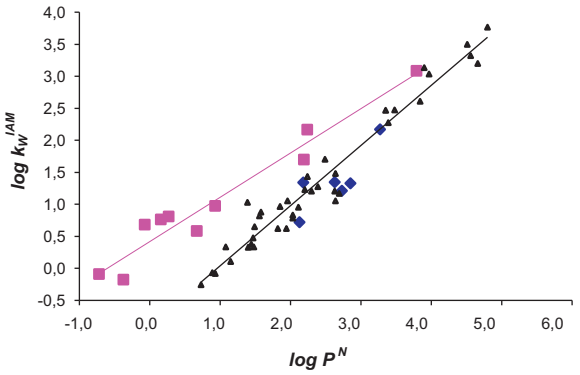

Fig. 3. Relationships between retention data on IAM.PC.DD2 measured by HPLC ($\log k_w^{IAM}$) and lipophilicity ($\log P^N$) for outliers (■), non-outliers (◆), and 39 neutral compounds (△).

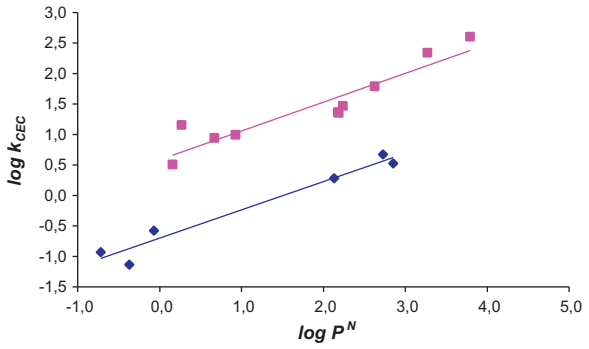

Fig. 4. Relationships between retention data on IAM.PC.DD2 measured by CEC ($\log k_{CEC}$) and lipophilicity ($\log P^N$) for protonated (■) and unprotonated (◆) analytes. Benzodiazepines are included in the subclass of protonated analytes.

thiopental) have $\log k_w^{IAM}$ values correctly predicted by the equation and they will be indicated as "non-outliers". The subset includes the compounds with $\log P > 1$, but benzodiazepines (oxazepam and temazepam).

The other subset, including the compounds with $\log k_w^{IAM}$ values higher than predicted by Eq. (3), includes benzodiazepines and all the molecules with $\log P < 1$; we indicated these compounds as "outliers". Interestingly, their behaviour does not depend on the protonation degree; indeed, the subset includes primary (phenylpropanolamine), secondary (atenolol, ephedrine), and tertiary (ranitidine) amines as well as molecules unionized at the experimental pH (caffeine and theobromine). This suggests that polar compounds can partition in phospholipids more strongly than expected on the basis of their lipophilicity, accordingly to the behaviour previously observed in partition studies in liposomes [20].

Finally, verapamil, the most lipophilic compound among those considered, is a border line case because it has a $\log k_w^{IAM}$ value only slightly higher than predicted by Eq. (3), which makes difficult its assignment to the first or the second subset. However, its inclusion in the subset of outliers significantly improves the regression equation between $\log k_w^{IAM}$ and $\log P^N$:

$$\underset{n=10}{\log k_w^{IAM}} = \underset{r^2=0.944}{0.691(\pm 0.059)}\ \log P^N + \underset{s=0.252}{0.420(\pm 0.096)} \quad (4)$$

Since this equation shows a lower slope value than Eq. (3), previously found for neutral compounds, the two regression lines are convergent.

CEC experimental data were used to calculate, as reported in Section 2, $\log k_{CEC}$ parameters. Since it is questioned whether, from a mechanistic point of view, a single electrophoresis parameter can be considered equivalent to the retention factor in HPLC [13], we investigated about $\log k_{CEC}$ effectiveness to express the interactions between analytes and phospholipids by studying the relationships with both $\log P^N$ and $\log k_w^{IAM}$ values.

The values of $\log k_{CEC}$ are reported in Table 1, whereas Fig. 4 shows the relationships between $\log k_{CEC}$ and $\log P^N$ values for the considered compounds.

As can be seen, the points lie on two different, almost parallel, lines (Fig. 4).

The relative relation equations are:

$$\underset{n=10}{\log k_{CEC}} = \underset{r^2=0.869}{0.473(\pm 0.065)}\ \log P^N + \underset{s=0.246}{0.585(\pm 0.142)} \quad (5)$$

$$\underset{n=6}{\log k_{CEC}} = \underset{r^2=0.959}{0.464(\pm 0.048)}\ \log P^N - \underset{s=0.177}{0.700(\pm 0.089)} \quad (6)$$

Eq. (5) refers to the line shifted upward in the graph. It is generated by benzodiazepines (oxazepam and temazepam) and all the compounds protonated at the experimental pH, i.e. atenolol, diphenhydramine, ephedrine, phenylpropanolamine, ketamine, ranitidine, tramadol, and verapamil.

Eq. (6) refers to the line shifted downward in the graph and it is generated by the compounds unprotonated at the experimental pH, but benzodiazepines, i.e. benzene, caffeine, nicotinamide, theobromine, thiopental, and toluene.

As can be seen, the two regression lines (Eqs. (5) and (6)) have practically the same slope, and the two lines are parallel. Therefore, for isolipophilic compounds, i.e. having the same $\log P^N$ value, the $\log k_{CEC}$ value can differ of about 1.3 units, depending on the occurrence or not of protonation. Analogously to the behaviour observed in HPLC, benzodiazepines are an exception because they fit the regression line of protonated analytes despite the fact they are unionized at the experimental pH. The high affinity for phospholipids of these compounds suggests that, beside polarity (in the cases of HPLC) and protonation (in the case of CEC) other structural, probably conformational, features can also enhance analyte/phospholipid affinity. However, further investigations on larger sets of compounds are needed to rationalize benzodiazepine behaviour.

It is interesting to note that the two subsets identified in CEC do not correspond to the subsets, outliers and non-outliers, identified in IAM-HPLC.

To better clarify the possible differences between CEC and HPLC data in the description of interactions between analytes and phospholipids, we also studied the relationships between the data achieved by CEC ($\log k_{CEC}$) and those achieved by HPLC ($\log k_w^{IAM}$). Actually, if the two data sets would encode the same intermolecular recognition forces between analytes and phospholipids (isodiscriminative behaviour), as expected for $\log k_{CEC}$ and $\log k_w^{IAM}$ values, they should be related by a single linear equation with a slope value near to unit and an intercept value near to zero. As can be seen in Fig. 5, where $\log k_{CEC}$ values are plotted against the corresponding $\log k_w^{IAM}$ values, the points are quite scattered and it is again evident that they describe two different lines.

All the compounds protonated at the experimental pH (i.e. atenolol, diphenhydramine, ephedrine, ketamine, phenylpropanolamine, ranitidine, and tramadol, but not verapamil) generate a line described by the following equation, which shows a slope value of about 1 and an intercept value near to zero:

$$\underset{n=7}{\log k_{CEC}} = \underset{r^2=0.835}{1.028(\pm 0.204)}\ \log k_w^{IAM} + \underset{s=0.269}{0.127(\pm 0.255)} \quad (7)$$

All the compounds unprotonated at the experimental pH (i.e. benzene, caffeine, nicotinamide, oxazepam, theobromine, temazepam, toluene, and thiopental) lie on a different line with a slope value slightly higher than unit and a negative intercept value;

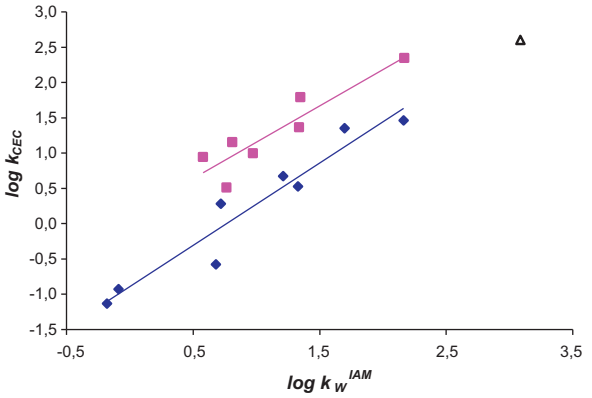

Fig. 5. Relationships between retention data on IAM.PC.DD2 measured by CEC ($\log k_{CEC}$) and by HPLC ($\log k_w^{IAM}$) for protonated (■) and unprotonated analytes (◆); verapamil is indicated as (Δ).

the relative relation equation is the following:

$$\underset{n=8}{\log k_{CEC}} = \underset{r^2=0.930}{1.168(\pm 0.130)\ \log k_w^{IAM}} \underset{s=0.283}{-0.892(\pm 0.158)} \quad (8)$$

Due to the different slope values, the two lines are convergent, making difficult the assignment of verapamil to the first or the second subset. However, the inclusion of verapamil in the second subset yields a new equation (Eq. (8a)) showing improved r^2 and s values:

$$\underset{n=9}{\log k_{CEC}} = \underset{r^2=0.960}{1.145(\pm 0.089)\ \log k_w^{IAM}} \underset{s=0.264}{-0.876(\pm 0.137)} \quad (8a)$$

The occurrence of two regression lines can be explained on the basis of the following reasons.

Considering the structure of IAM.PC.DD2 stationary phase, where both negative and positive charges are present (on phosphate and choline group, respectively), the retention of protonated compounds arises from a concerted mechanism including both lipophilic and electrostatic interaction forces. Although in a first attempt to transfer HPLC method to the CEC system, we tried to use the same buffer used in HPLC (100 mM phosphate), during electrochromatographic runs, a high current was recorded, and we had to change the type of buffer, selecting a 10 mM Tris–HCl buffer. Different buffer composition, as well as different ionic strength [5,21], can modulate differently electrostatic interactions of charged analytes with phospholipids, which can explain the increase of retention factors of positively charged analytes in CEC with respect to HPLC.

4. Conclusion

The results of this work suggest that CEC is an effective technique to measure drug/phospholipid interactions.

Analogously to IAM-HPLC data, CEC data are not collinear to lipophilicity, as it is expressed by *n*-octanol/water log*P* values, because positively charged analytes are retained stronger than unprotonated isolipophilic compounds. This suggests that retention of cations takes into account the occurrence of electrostatic intermolecular recognition forces. Nevertheless, retention of neutral and charged species is directly proportional to *n*-octanol lipophilicity, but only if two subsets, i.e. protonated and unprotonated analytes, are considered separately.

The main difference between CEC and HPLC data arises from the fact that the presence of a positive charge on an analyte affects phospholipid affinity more strongly in CEC than in IAM-HPLC, probably due to the reduction of salt concentration in CEC eluents, operated to avoid high current. However, the electrostatic component of interaction with phospholipids decreases at increasing lipophilicity making less evident the differences between CEC and HPLC retention for the most lipophilic analytes.

This work indicates the potential of CEC technique for application in the determination of drug/phospholipid affinity because, although a preliminary screening between protonated and unprotonated analytes is requested, linear relationships are observed between CEC and HPLC data. Although CEC is more complex than HPLC, because phospholipid interaction must be extrapolated from experimental data also encoding further and concomitant retention mechanisms, it is a faster technique than HPLC (about 80% time saved for the most lipophilic analytes) and requires much less amount of analyte, eluent, and stationary phase to be performed. Moreover, CEC elution with 100% aqueous eluent was possible for the vast majority of analytes (13 out of 16), whereas it was possible for only five compounds in HPLC. This is very advantageous in industrial field because early screening of new drug-candidate pharmacokinetics can be only performed by high-throughput methods.

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