Journal of Pharmaceutical and Biomedical Analysis 55 (2011) 409–413

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

# A quantitative structure–activity approach for lipophilicity estimation of antitumor complexes of different metals using microemulsion electrokinetic chromatography

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ARTICLE INFO

*Article history:*
Received 27 October 2010
Received in revised form 9 February 2011
Accepted 10 February 2011
Available online 17 February 2011

*Keywords:*
Anticancer drugs
Gallium(III) complexes
Iron(III) complexes
Lipophilicity
Microemulsion electrokinetic chromatography

ABSTRACT

Microemulsion electrokinetic chromatography (MEEKC) offers a valuable tool for the rapid and highly productive determination of lipophilicity for metal-based anticancer agents. In this investigation, the MEEKC technique was applied for estimation of *n*-octanol–water partition coefficient ($\log P_{oct}$) of a series of antiproliferative complexes of gallium(III) and iron(III) with $^4N$-substituted α-*N*-heterocyclic thiosemicarbazones. Analysis of relationships between the experimental $\log P_{oct}$ and the retention factors of compounds showed their satisfactory consistency in the case of single metal sets, as well as for both metals. Since none of available calculation programs allows for evaluating the contribution of central metal ion into $\log P_{oct}$ (i.e. $\Delta\log P_{oct}$) of complexes of different metals, this parameter was measured experimentally, by the standard 'shake-flask' method. Extension of the $\log P_{oct}$ programs by adding $\Delta\log P_{oct}$ data resulted in good lipophilicity predictions for the complexes of gallium(III) and iron(III) included in one regression set. Comparison of metal-thiosemicarbazonates under examination in terms of $\log P_{oct}$ vs. antiproliferative activities (i.e. 50% inhibitory concentration in cancer cells) provided evidence that their cytotoxic potency is associated with the ability to cross the lipid bilayer of the cell-membrane via passive diffusion.



## 1. Introduction

Lipophilicity is an essential factor in drug development equation as it determines important biological processes accompanying the intake of a drug such as absorption, transport through membranes, drug–receptor interactions. Therefore, pharmaceutical companies are paying much attention on the selection of developmental compounds with high in vivo permeability at the earliest possible opportunity. This issue appears to be especially important in the case of metal-based antitumor agents whose discovery and clinical development remain fairly inefficient. The ability of a potential drug candidate to penetrate biomembranes (until it binds to the target and induces the desired response) is most often measured using octanol–water partition. As a widely used lipophilicity parameter in medicinal chemistry, the octanol–water partition coefficient ($\log P_{oct}$) can be determined for metal coordination compounds as potential drugs by use of the shake-flask method [1–4], HPLC [5,6], different $\log P_{oct}$ estimation software [2,7] or quantum chemical approaches [8,9]. However, none of these tools, until now focused mostly on platinum(II) complexes, seems to be satisfactory for such bioavailability testing. Indeed, experimental determination of $\log P_{oct}$ is often a challenge because of intricate behavior of metal-ligand compounds in liquid–liquid extraction and reversed-phase HPLC systems. On the other hand, standard programs for $\log P_{oct}$ prediction are not parameterized for metal complexes. Attempts to calculate their $\log P_{oct}$ by using quantum chemical methods are often failed with regard to assessing the input of central metal atom. As a result, calculation may provide poor predictions.

MEEKC presents an alternative approach to resolve the problem of accurate lipophilicity estimation for metal complexes. In this basically electromigration separation technique, the behavior of analytes is governed by their different distributions between an essentially aqueous electrolyte and microdroplets of an organic, water-immiscible solvent stabilized by a charged surfactant. It

*Abbreviations:* MEEKC, microemulsion electrokinetic chromatography; $\log P_{oct}$, *n*-octanol–water partition coefficient; $\Delta\log P_{oct}$, difference between $\log P_{oct}$ values for metal complex and ligand; $\log P^{c}_{oct}$, calculated value of $\log P_{oct}$; $\log P^{L}_{oct}$, partition coefficient of a ligand; $\log k$, retention factor; $IC_{50}$, 50% inhibitory concentration in cancer cells.

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doi:10.1016/j.jpba.2011.02.011

stands to reason that such dispersed, oil-like phase may play a role of the solubilizing medium, mimicking the interior part of biological membranes, as *n*-octanol does. MEEKC was shown to provide a good experimental basis for the indirect assessment of lipophilicity of a number of antitumor complexes of platinum(II) [10] and gallium(III) [11], which is based on linear regression of retention factors ($\log k$) against experimental or calculated $\log P_{oct}$ ($\log P^{c}_{oct}$). It should be noted that due to the absence of genuine (chromatographic) stationary phase MEEKC conditions are much friendlier with respect to metal complexes than HPLC ones, thus maintaining the solute integrity in the separation system. To this point, the recognized advantages of HPLC, such as high productivity, low sample consumption, and insensitivity to impurities, are all preserved in MEEKC.

In the present study, our efforts were directed toward extension of the bottom-up MEEKC methodology to the challenging case of *different* central metal complexes, by considering thiosemicarbazonates of gallium(III) and iron(III) known as potent antiproliferative agents [12,13]. Recently synthesized in our group these compounds have not been characterized in terms of $\log P_{oct}$ yet and this bottleneck retards their further development. While collecting and correlating $\log k$ and experimental $\log P_{oct}$ data were quite straightforward, the exploitation of $\log P^{c}_{oct}$–$\log k$ relationships required extension of existing $\log P_{oct}$ methods (basically developed without any metal-containing compounds). This was done by incorporating lipophilicity data for metals ($\Delta \log P_{oct}$) obtained from experiment.

## 2. Materials and methods

### 2.1. Materials

All reagents and solvents were obtained from commercial suppliers and used as received. The thiosemicarbazone complexes (see Fig. 1 for structural formulas) were synthesized as described in the literature (note that one of gallium compounds, **1F**, belongs to a semicarbazonate class) [12,14]. Manganese(III) porphyrinate used as a marker of microemulsion droplets was a courtesy of Prof. Elena Milaeva (Moscow State University).

### 2.2. Instrumentation

All MEEKC experiments were performed with a CAPEL 105 M instrument (Lumex, St. Petersburg, Russia) controlled by an Elphoran software, and polyimide-coated fused-silica capillary (from BGB Analytik, Schlossboeckelheim, Germany) of 75-μm i.d. with a length of 40.5 cm, 31.5 cm to the detector. Detection was carried out using a build-in variable wavelength UV detector operating at 200 nm.

### 2.3. Procedures

#### 2.3.1. Measurement of retention factors

All separations were performed with the capillary thermostated at 25 °C, and the voltage was applied as a positive potential to the inlet vial. Additionally, the hydrodynamic pressure (20 mbar) was applied at the inlet vial to shorten the analysis time. Injections were performed by placing the sample in the inlet vial and applying pressure (20 mbar) for a designated time.

Prior to use, a new capillary was conditioned with 1 M NaOH for 30 min and daily with 0.1 M NaOH for 15 min, water for 1 min, acetone for 2 min, water for 1 min, and finally with microemulsion (10 min). Between separations, the capillary was conditioned with 0.1 M NaOH and water (1 min each) and then with microemulsion for 3 min. Each 3 runs both vials were replenished with a fresh microemulsion to guarantee repeatable migration times.

Microemulsion was prepared by dissolving 0.288 g SDS in a mixture of 4 ml 10 mM phosphate buffer (pH 7.4), 0.2 ml *n*-heptane and 1.6 ml *n*-butanol, sonication for 5 min in an ultrasonic bath, addition of 14.4 ml 10 mM phosphate buffer, further sonication for 30 min, and finally modification with isopropanol [11]. Samples were prepared as a 1 g $l^{-1}$ solution in acetone and diluted (1:10) with microemulsion.

The method development implied the optimization of several factors, including isopropanol concentration, sample volume, applied voltage, and hydrodynamic pressure. The optimized set of variables comprised 10% (v/v) organic modifier, 200 mbar × s injections, and 22 kV and 10 bar as separation voltage and pressure, respectively, and resulted in a standard deviation of 0.003 units of $\log k$.

The retention factor for neutral and charged complexes was calculated from Eqs. (1) and (2), respectively [11]:

$$k = \frac{t - t_{eof}}{t_{eof}(1 - (t/t_{me}))} \quad (1)$$

where $t$, $t_{eof}$, and $t_{me}$ represent the migration times of the solute, the EOF (given by an acetone marker), and the oil droplet, respectively.

$$k = \frac{\mu - \mu_{eph}}{\mu_{me} - \mu} \quad (2)$$

where $\mu$ is the electrophoretic mobility of the solute in the MEEKC system, $\mu_{eph}$ is the electrophoretic mobility of the solute when the pseudostationary phase is not present (obtained under CZE conditions), and $\mu_{me}$ is the electrophoretic mobility of the oil droplet.

#### 2.3.2. Measurement of $\log P_{oct}$

Weighted amounts of compounds were partitioned between equal volumes of water and *n*-octanol for 2 h at room temperature using an automatic shaker. Metal concentrations in the aqueous phase before ($C_0$) and after partitioning ($C_{aq}$) were measured using inductively coupled plasma atomic emission spectroscopy (higher concentrations) or inductively coupled plasma mass spectrometry (lower contents). The partition coefficient was calculated as the logarithm of the concentration ratio of the substance distributed in the biphasic system using the following equation:

$$\log P_{oct} = \log \frac{C_0 - C_{aq}}{C_{aq}} \quad (3)$$

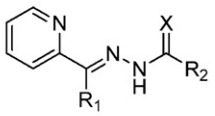
Analytical measurements were performed at the Institute of Microelectronics Technology and High-Purity Materials, Russian Academy of Sciences (Chernologolovka, Russia) (see Supporting Information for instrumental parameters; Tables S-1 and S-2).

### 2.4. Computation

Experimental $\log k$ data were submitted to a linear regression analysis against $\log P_{oct}$ and $\log P^{c}_{oct}$. In the latter case, eleven programs were employed without modification (Table S-4) to predict $\log P_{oct}$ of a ligand ($\log P^{L}_{oct}$), from which $\log P^{c}_{oct}$ of metal complexes were calculated using experimental $\Delta \log P_{oct}$ values as described below. The regression equations derived were tested according to the requirements of a meaningful correlation analysis by considering linear Pearson (squared) correlation coefficient, $R^2$, the standard deviation of fit, $s$, the cross-validated $R^2$ value, $R^2_{CV}$, and mean absolute error of estimate as a selection of measures of the fit to assess the overall accuracy of prediction. All statistical analysis employed the SPSS Statistics 17.0 package (IBM, NY, USA).

## 3. Results and discussion

Fig. 2 shows typical chromatograms for MEEKC analysis. The $\log k$ values were calculated as described in Section 2.3.1 and are



$HL^A$: X = S, $R_1$ = $CH_3$, $R_2$ = $NH_2$

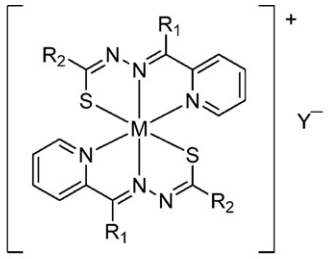
$HL^B$: X = S, $R_1$ = $NH_2$, $R_2$ = $NH_2$

$HL^C$: X = S, $R_1$ = $CH_3$, $R_2$ = $N(CH_3)_2$

$HL^D$: X = S, $R_1$ = $NH_2$, $R_2$ = $N(CH_3)_2$

$HL^E$: X = S, $R_1$ = $CH_3$, $R_2$ = $NHC_6H_5$

$HL^F$: X = O, $R_1$ = $CH_3$, $R_2$ = $N(CH_3)_2$



M = Ga, L = $L_A$, Y = $NO_3$; $[Ga(L_A)_2][NO_3]$ (1A)

M = Ga, L = $L_B$, Y = $NO_3$; $[Ga(L_B)_2][NO_3]$ (1B)

M = Ga, L = $L_C$, Y = $PF_6$; $[Ga(L_C)_2][PF_6]$ (1C)

M = Ga, L = $L_D$, Y = $NO_3$; $[Ga(L_D)_2][NO_3]$ (1D)

M = Ga, L = $L_E$, Y = $PF_6$; $[Ga(L_E)_2][PF_6]$ (1E)

M = Ga, L = $L_F$, Y = $PF_6$; $[Ga(L_F)_2][PF_6]$ (1F)

M = Fe, L = $L_A$, Y = $NO_3$; $[Fe(L_A)_2][NO_3]$ (2A)

M = Fe, L = $L_B$, Y = $NO_3$; $[Fe(L_B)_2][NO_3]$ (2B)

M = Fe, L = $L_C$, Y = $PF_6$; $[Fe(L_C)_2][PF_6]$ (2C)

M = Fe, L = $L_D$, Y = $NO_3$; $[Fe(L_D)_2][NO_3]$ (2D)

M = Fe, L = $L_E$, Y = $PF_6$; $[Fe(L_E)_2][PF_6]$ (2E)

**Fig. 1.** The collection of thiosemicarbozanate complexes of gallium and iron under investigation.

assembled in Table S-3 (see Supporting Information). The retention mechanism involved for metal complexes in MEEKC has shown to be depended on their actual charge state in the microemulsion environment [7]. While for neutral complexes dominating are hydrophobic interactions with oil-droplets, the affinity of the charged complexes toward oil-droplet pseudostationary phase has a more complex character. Along with hydrophobic mechanism, the electrostatic interaction between cationic solutes and anionic SDS surfactant stabilizing the microemulsion may contribute into retention. The metal complexes studied exist in the microemulsion medium both as neutral and as positively charged species (Table S-3). For instance, of gallium complexes, **1B**, **1E**, and **1F** occur in the form of intact uncharged ion-pairing associates, $[ML_2]Y$, whereas **1A**, **1C**, and **1D** undergo dissociation that leads to the formation of cationic chelates, $[ML_2]^+$.

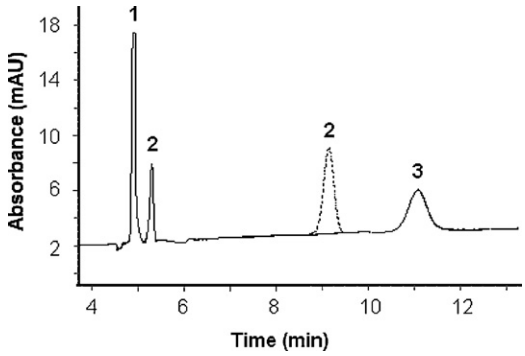



**Fig. 2.** Superimposition of MEEKC signals of two thiosemicarbozanate complexes of different lipophilicity. Peak identification: **1** – acetone (marker of the electroosmotic flow); **2** – **2A** (solid line) or **2C** (dashed line); **3** – manganese(III) porphyrinate (marker of microemulsion droplets). MEEKC conditions, see Section 2.

Regardless of the charge of a solute, its $\log P_{oct}$ as a lipophilicity indicator can be determined by MEEKC from the relationship with $\log k$ through a linear function as given in the following equation [15]:

$$\log P_{oct} = a \log k + b \quad (4)$$

where $a$ and $b$ are regression coefficients. It should be underlined that the validity of Eq. (4) is due to free-energy similarities in solute partitioning in octanol and oil-droplet phase. Regressions of $\log P_{oct}$ against $\log k$ resulted in acceptable accuracy ($R^2 > 0.98$) for each group of compounds under scrutiny, i.e. complexes of gallium and iron. Furthermore, combining both metal complexes in a single dataset yielded a pleasing fit, with squared regression coefficient $R^2 = 0.979$ and standard deviation $s = 0.15$ (the largest absolute error was 0.30 log unit). It is encouraging to point out the structural variety of compounds used to construct this regression (as shown in Fig. 1), including both Ga(III) and Fe(III) complexes, a variety of thiosemicarbazone ligands, different counter-ions and last but not least of all, net charge (see above). In order to emphasize the predictive ability of Eq. (4), it was tested with two additional gallium(III) thiosemicabazonates used at the stage of optimizing MEEKC conditions and hence having the known $\log k$. Calculated $\log P_{oct}$ were found to be in a satisfactory agreement with experimental values determined in our preceding work [2], with a mean absolute error of 0.16.

Although the model operating with Eq. (4) is both physically realistic and statistically valid, its practical implementation requires the knowledge of the experimental set of $\log P_{oct}$ to adjust the parameters $a$ and $b$. As mentioned above, for metal complexes measurement of the distribution property might be quite challenging (let alone its cost and time consumed); for instance, reliable partition data were not accessible here for all of the tested compounds (cf. Table S-3 in the Supporting Information and Fig. 1).

**Table 1**
Statistics of linear regressions of calculated $\log P_{oct}$[a] against $\log k$ of metal complexes with hexafluorophosphate counter-ions.

| Program | $R$ | $R^2_{CV}$ | $s$ |
|---|---|---|---|
| ChO 11.0 LogP | 0.921 | 0.960 | 0.45 |
| ChO 11.0 CLogP | 0.860 | 0.927 | 1.13 |
| AlogPs | 0.976 | 0.988 | 0.32 |
| AC logP | 0.999 | 0.999 | 0.04 |
| AB/LogP | 0.980 | 0.990 | 0.23 |
| milogP | 0.860 | 0.927 | 0.87 |
| ALOGP | 0.991 | 0.996 | 0.18 |
| KOWWIN | 0.957 | 0.978 | 0.69 |
| Xlogp2 | 0.971 | 0.985 | 0.33 |
| Xlogp3 | 0.996 | 0.998 | 0.12 |
| COSMOFrag | 0.957 | 0.978 | 0.57 |

[a] $\Delta\log P_{oct}$ −3.56 and −4.01 for Ga(III) and Fe(III), respectively.

Therefore, in order to further examine the applicability of MEEKC to accurately assess the lipophilicity of structurally diverse metal-based drugs, we have applied a computational approach to generate $\log P^c_{oct}$ suitable for a more extensive regression treatment. A representative selection of programs obtainable from the literature (information regarding their brief characteristics and Internet source is provided in Table S-4) was utilized for prediction of $\log P^c_{oct}$ of metal complexes. Unsurprisingly, no one model provided good correlations ($R^2 < 0.5$), since either databases employed in developing the programs did not contain any metal-containing compounds, or the programs were trained with complexes of a single type of metal [3,8]. A two-step strategy to overcome this limitation adopted here was to calculate the increment of a metal ion into $\log P_{oct}$ as [16]

$$\Delta\log P_{oct} = \log P_{oct} - 2\ \log P^L_{oct} \quad (5)$$

where $\log P^L_{oct}$ were taken from Ref. [13] and accumulation factor 2 reflects the stoichiometry of the complexes under consideration (see Fig. 1). The mean value of $\Delta\log P_{oct}$ was averaged with respect to all available complex/ligand combinations for a given metal and then used as metal descriptor. The latter was summed with the calculated $\log P^L_{oct}$ in an additive manner to produce an extended set of $\log P^c_{oct}$.

However, none of the extended programs gave satisfying fittings for the entire set of complexes, being applicable only to a specific Y group in compounds of the type $[ML_2]Y$. Within subsets of complexes with the same counter-ion the situation is much more favorable, as can be seen from the statistical results for lipophilicity prediction of gallium and iron complexes compared for different programs in Table 1. For the sake of conciseness, correlations performed with a dataset involving the hexafluorophosphate complexes are only included in the table. For the nitrate compounds, the statistical goodness of predictions is notably inferior: consistent approximations were only obtained using 4 of 11 programs (see for detail Table S-5 in the Supporting Information).

The best fit of $\log P^c_{oct}$ for metal complexes with $PF_6^-$ as counter-ion was obtained using AC LogP program as shown below:

$$\log P^c_{oct} = 9.24(\pm0.13)\log k - 9.26(\pm0.12) \quad (6)$$

($R^2 = 0.9996$; $s = 0.04$; values in parentheses are standard errors of regression at 95% confidence limit).

Regarding pharmaceutical implications of our lipophilicity testing, certain forecast for the behavior of metal-thiosemicabazonate complexes in vivo can be done. Only two compounds, **1C** and **2C**, exhibit a positive $\log P_{oct}$ value indicating a fairly high lipophilicity. Other complexes are essentially hydrophilic and would probably face lower transport to the cells and greater accumulation in the blood plasma. We attribute such properties to the fact that the ligand hydrophobicity itself matters not as much as the strength of the ion pair and thereby an actual charge of the complex. However, yet the most hydrophilic compound, **2A**, cannot be ruled out from further evaluation (for the reason of low membrane permeability), since a clinically approved platinum drug, oxaliplatin, displays exactly the same distribution property ($\log P_{oct}$ – 1.65 [17]). The comparison of the acquired $\log P_{oct}$ values and the data of in vitro growth inhibition against human tumor cells (50% inhibitory concentration, $IC_{50}$) [12,14], as shown in Table 2, revealed no straightforward correlation between lipophilicity and cytotoxicity. Rather a parabolic relation exists, usual for compounds disclosing their biological activity inside the cell [18], that is, the lowest $IC_{50}$ exhibited metal complexes with intermediate lipophilicity that facilitates crossing the cellular membrane. For instance, the most active compounds against breast cancer cell line SK-BR-3 in the series of gallium and iron complexes with increasing distribution are **1D** and **2D**, respectively (see Table 2).

**Table 2**
Lipophilicity versus cytotoxicity of metal-thiosemicarbazonates.

| Compound | $\log P_{oct}$ | $IC_{50}$ (nM)[a] |
|---|---|---|
| **1A** | −0.57 | 1.0 ± 0.1 |
| **1B** | −1.06 | 3.4 ± 1.0 |
| **1D** | −0.93 | 0.11 ± 0.05 |
| **1F** | −1.36 | >1000 |
| **2A** | −1.65 | 28 ± 12 |
| **2B** | −0.35 | >100 |
| **2C** | 0.27 | >100 |
| **2D** | −0.90 | 1.6 ± 0.7 |

[a] In SK-BR-3 human cancer cell line [12,14].

## 4. Conclusions

In summary, the results of this quantitative structure–activity study revealed that MEEKC method can be used to obtain good estimates of octanol–water partition coefficients of antiproliferative complexes differing in metal identity. On the other hand, application of several $\log P_{oct}$ methods to predict lipophilicities of different metal complexes indicated low accuracy of predictions using existing calculation programs. A substantial improvement of computational approach in terms of predictive power was achieved by incorporating the increment of central metal taken from experimental $\log P_{oct}$ data for given metal complexes and respective ligands. In vitro antiproliferative activity of gallium(III) and iron(III) thiosemicabazonates was found to be parabolically related with lipophilicity.

## Acknowledgement

The authors thank Dmitriy Grozdov (Vernadsky Institute) for valuable assistance with statistical analysis.

## Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jpba.2011.02.011.

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