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Application of the ion pair concept to the *n*-octanol–water partitioning of cefepime and cefpirome

S.-A. Koufopoulou, C. Pistos, C. Giaginis, A. Tsantili-Kakoulidou *

Department of Pharmaceutical Chemistry, School of Pharmacy, University of Athens, Panepistimiopolis, Zografou, Athens 157 71, Greece

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Abstract

The ion pair concept was applied for the assessment of lipophilicity of cefepime and cefpirome. Octanol–water distribution coefficients were determined in presence of different concentrations [X^-] of sodium octanesulphonate. The log D_x values within the linear part of the log D_x/[X^-] relationships were extrapolated to log D_o values corresponding to the partitioning in absence of the counter ion. Measurements were feasible at pH values close to the isoelectric points of the acidic and basic functions. In that pH range the conduction of the experiments in presence of the hydrophobic counter anion facilitated the partitioning of the two cephalosporins to octanol, circumventing the problems arising from their high hydrophilicity. This procedure could not be applied at lower pH, possibly due to a further drastic decrease in the 'intrinsic' lipophilicity or to reduced ion pairing potential of octanesulphonate, and at higher pH due to the disruption of the zwitterionic structure. Extrapolated log D_o values were compared to actual log D measurements performed for a reference quinolinium compound and for cefpirome. Extrapolated retention factors log k_w close to the isoelectric point were also determined by reversed phase HPLC and compared to the log D_o values.

Keywords: Cefepime; Cefpirome; Zwitterion; Ion pair; Octanol–water partitioning; Liquid chromatography

1. Introduction

Cefepime (Cef) and Cefpirome (Cefp) are fourth generation zwitterionic cephalosporins with similar chemical structures (Fig. 1). They are highly hydrophilic and have to be administered intravenously or intramuscularly because of their insufficient bioavailability (Harman et al., 1996). They possess acidic pK_a values 1.12 and 1.62 and basic pK_a values, corresponding to the aminothiazole moiety, 3.07 and 3.11, respectively (Mrestani et al., 1998; Evagelou et al., 2003). Zwitterionic species formation may be possible between the two opposite charged centers at the pH corresponding to isoelectric points, around 2.0 and 2.3, respectively. Of physiological relevance however is the zwitterionic species formed between the carboxylate anion and the positively charged quaternary nitrogen, which stabilizes lipophilicity over a broad pH range. This behavior was reflected in the reversed phase HPLC retention profile of the two cephalosporins, previously reported (Pistos et al., 2003). The predominance of the zwitterionic species at physiological pH may be responsible for the high diffusion rates of Cef and Cefp through the porin channels of the outer membrane surrounding the gram-negative bacteria (Nikaido et al., 1990), a process favored by the high hydrophilicity of the drugs. An early attempt to correlate the lipophilicity of cephalosporins, expressed as octanol–water partition coefficients, log P, to their permeability rate through the porins, revealed a negative relationship in the case of anionic compounds, while this effect was not so evident for the zwitterionic analogues (Yoshimura and Nikaido, 1985). It should be noted that that study included mainly cephalosporins with lipophilicity high enough, to be experimentally determined.

For highly hydrophilic drugs like Cef and Cefp direct partitioning experiments using the traditional shaking flask method present difficulties, due to their high affinity for the aqueous phase. The use of reversed phase liquid chromatography, a popular alternative for lipophilicity assessment, although well standardized for neutral and basic drugs, is associated with drawbacks in the case of acidic or zwitterionic compounds (Lombardo et al., 2001; Pistos et al., 2005). Moreover the presence of permanently charged centers in the molecules renders log P estimation

* Corresponding author. Tel.: +30 210 7274530; fax: +30 210 7274747.
E-mail address: tsantili@pharm.uoa.gr (A. Tsantili-Kakoulidou).

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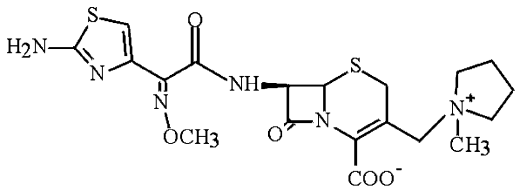
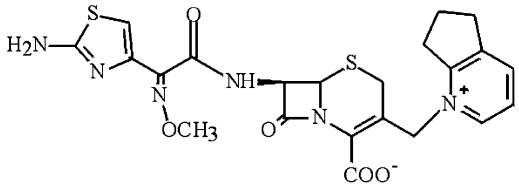


Cefepime.2HCl.H2O



Cefpirome.H2SO4

Fig. 1. Chemical Structures of cefepime and cefpirome.

by the available calculation systems less reliable (Hansch and Leo, 1995).

On the other hand charged molecules can form ion pairs with hydrophobic counter ions. This association leads to a considerable increase in their lipid solubility facilitating their extraction procedure and improving their absorption through membranes (Green et al., 1989; Adjei et al., 1993; Quintanar-Guerrero et al., 1997; Hatanaka et al., 2000; Sarveiya et al., 2004). Endogenous substances like prostaglandins, bile acids and eventually phospholipids, may also act as counter anions to form hydrophobic ion pairs with positively charged drugs, influencing their biological properties (Austin et al., 1995; Takacs-Novak and Szasz, 1999; van Balen et al., 2001). Less investigated is the effect of hydrophobic counter ions on zwitterionic compounds. In a recent publication it is reported that bile salts acted as enhancers of the lipophilicity and thereupon of the absorption of cefpirome (Mrestani et al., 2003). In contrast, according to our previous findings concerning the RP-HPLC retention profiles of Cef and Cefp, the addition of ion pairing reagents in the mobile phase produced a strong decrease in retention, which was attributed to the disruption of the zwitterionic charge compensation and the deliberation of a net charge in the molecules (Pistos et al., 2003).

In the present study we applied the ion pair concept in the octanol–water partitioning of Cefepime and Cefpirome with the main objectives to (i) to monitor the action of counter anions as lipophilicity modulators at different pH and compare it to their effect on the retention profile in reversed phase ion pair HPLC (Pistos et al., 2003) and (ii) to establish relationships between the apparent lipophilicity and the counter anion concentration and use them in order to determine the 'intrinsic' octanol–water distribution coefficient ($\log D_o$) by extrapolation. Moreover in order to be able to put side by side the 'intrinsic' octanol–water distribution coefficients with the corresponding reversed phase retention data we extended our previous HPLC investigation by determining the extrapolated retention factors $\log k_w$. To this end we should note that in the text the term partition coefficient ($\log P$) is used for 'true' lipophilicity, while the more general term distribution coefficient ($\log D$) is used for 'apparent' lipophilicity which depends on experimental conditions (pH, counter anion concentration).

2. Materials and methods

Cefepime dihydrochloride monohydrate (Cef) was provided by Bristol-Myers Squibb. Cefpirome sulfate (Cefp) was provided by Aventis. Compound CGX-0057364 was kindly donated by COMGENEX (Fig. 2) and was used as a reference substance in order to evaluate the effect of the counter anion in the final $\log D_o$ value.

Solvents of HPLC grade, octanol (extra pure) and sodium octanesulphonate (analytical grade) were purchased from Lab-Scan Analytical Sciences Ltd., Ireland. Water was deionised and further purified by means of a Milli-Q Plus water purification system (Millipore Co., USA).

2.1. Measurement of distribution coefficients

Octanol/water partition experiments were performed by the shaking flask method (Vrakas et al., 2003) using the following experimental conditions.

For compound CGX-0057364 the aqueous phase consisted of pure water or of water containing various concentrations of sodium octanesulphonate in a range $0.7–5.0 \times 10^{-4}$ M. The aqueous and organic phases were mutually saturated before the experiment. The compound was dissolved in the aqueous phase at concentrations 1.0, 2.5 and 5.0×10^{-5} M. The phase volume ratio V_{aq}/V_{oct} was 15:2 (ml) or 20:2 (ml) in the case of higher counter ion concentration. For Cefepime and Cefpirome sodium octanesulphonate was added in the aqueous phase at a concentration range 0.005–0.035 M and the pH was appropriately adjusted by phosphoric acid 25%. Cef and Cefp were dissolved in the aqueous phase at concentrations 2.5 and 5.0×10^{-5} M. The phase volume ratio V_{aq}/V_{oct} was 5: 40 (ml). In each case the two phases were equilibrated for 90 min at 25 °C in thermostatic vials. Centrifugation followed for 30 min at 2500 rpm. The aqueous phase was analysed before and after equilibration by HPLC applying the conditions described under 2.2. Distribution coefficients were calculated according to Eq. (1):

$$D = \frac{A_o - A_1}{A_1} \times \frac{V_{aq}}{V_{oct}} \quad (1)$$

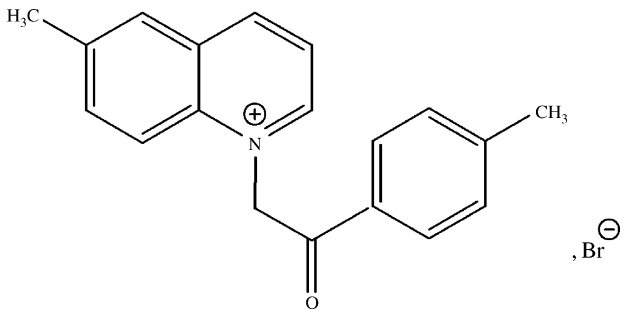


Fig. 2. Chemical Structure of compound CGX-0057364.

A_o and A_1 being the peak area before and after equilibration, respectively.

For Cefpirome the same experiments were performed also in absence of octanesulphonate, using 100 mM phosphate buffer pH 2.5 and 100 mM phosphate buffer pH 7.4 as the aqueous phase. The phase volume ratio was 1:40 and the equilibration time was 12 h.

Each experiment was performed at least in triplicate. The standard deviation did not exceed ±0.07 log units.

2.2. HPLC analysis

The HPLC system consisted of a Waters model 501 pump, equipped with a Waters Model 486 variable wavelength UV–vis detector (8 μl flow cell). The injection system was Rheodyne Model 7125 with a 5 μl loop. The effluent was monitored at 260 nm. The stationary phase was an ODS column (25 cm × 4 mm i.d.) pre-packed with Lichrosorb RP-18 (particle size 10 μm). The mobile phase consisted of a mixture of methanol/100 mM phosphate buffer 20/80 adjusted at pH 3.0. In the case of compound CGX-0057364 a methanol/phosphate buffer mixture 60/40 at pH 3.0 was used. Each sample was injected at least four times and the mean Peak Area was obtained with R.S.D.% ranging between 0.94 and 1.14%.

2.3. Determination of HPLC capacity factors

For the determination of capacity factors methanol and acetonitrile were used as organic modifiers in four different concentrations ranging from 10 to 25%. The aqueous component of the mobile phase was 100 mM phosphate buffer adjusted at pH 2.5. Retention times t_r were recorded and converted to $\log k$ via Eq. (2):

$$\log k = \log\left[\frac{t_r - t_o}{t_o}\right] \quad (2)$$

t_o being the retention time needed for the eluent to pass through the column. It was measured as a perturbation peak of the baseline.

Measurements were performed at least in duplicate for each organic modifier concentration.

2.4. Calculation of partition coefficients

Partition coefficients of Cefepime, Cefpirome and of compound CGX-0057364 were calculated according to two software packages: ClogP v.4.0, Biobyte Corp and Pallas for Windows version 3.1.1.2 using the Artificial Neural Network (ANN) option of the PrologP 7.0 module. The estimated values are reported in Table 1.

Table 1
Calculated log *P* values for cefepime, cefpirome and CGX-0057364

Compound	ClogP	PrologP (ANN)
Cef	−2.89	−2.79
Cefp	−2.62	−2.61
CGX-0057364	1.085	0.68

3. Results

3.1. Octanol–water partitioning of compound CGX-0057364 in presence and absence of octanesulphonate

Compound CGX-0057364 was preliminary used in order to evaluate the application of the ion pair concept in the assessment of lipophilicity. Compound CGX-0057364 (Fig. 2) contains a positively charged quaternary nitrogen atom capable to form ion pair with octanesulphonate, while due to the absence of ionisable centres no other equilibriums are taking place. Moreover, its log *P* value, initially estimated by calculation (Table 1), could reliably be measured. Partitioning experiments were performed in presence and absence of sodium octanesulphonate. The concentration of the counter anion was in the range of 10^{-4} M. Using three different compound concentrations in a tenfold lower range for each counter anion concentration, no concentration effect in the measured distribution coefficients was observed. A linear relationship between the logarithm of distribution coefficient $\log D_x$ and the counter anion concentration $[oct^-]$ was observed, which permitted extrapolation to $\log D_o$, according to Eq. (3).

$$\log D_x = S\,[oct^-] + \log D_o \quad (3)$$

The subscript x denotes the counter anion concentration. Log D_o corresponds to the distribution coefficient in absence of the counter ion. The slope S reflects the change in $\log D_x$ per octanesulphonate concentration unit. A satisfactory correlation coefficient was obtained, while $\log D_o$ value was found to be close to the directly measured partition coefficient $\log D_{exp}$ with a positive difference $\Delta = 0.24$, indicating a small effect of the counter anion in the extrapolated value (Table 2).

3.2. Octanol–water partitioning of cefepime and cefpirome in presence of octanesulphonate

Due to the high hydrophilicity of the drugs increased concentration of sodium octanesulphonate were required in order to permit an adequate increase in apparent lipophilicity, while

Table 2
Extrapolated $\log D_o$, slopes S, correlation coefficients and directly measured $\log D_{exp}$

Compound	$\log D_o$	S	r	$\log D_{exp}$
CGX-0057364	0.74 (±0.05)	1186.7 (±154.6)	0.983	0.50 (±0.03)
Cefepime				
pH 1.5	−3.11 (±0.10)	68.00 (±4.78)	0.993	
pH 2.0	−2.62 (±0.12)	60.40 (±6.74)	0.988	
pH 2.5	−2.79 (±0.06)	35.20 (±2.62)	0.994	
Cefpirome				
pH 1.5	−2.29 (±0.06)	77.60 (±3.98)	0.996	
pH 2.0	−2.12 (±0.06)	71.80 (±4.13)	0.997	
pH 2.5	−2.06 (±0.06)	49.00 (±0.58)	0.999	−2.30(±0.04)
pH 3.0	−2.36 (±0.04)	21.60 (±1.98)	0.992	

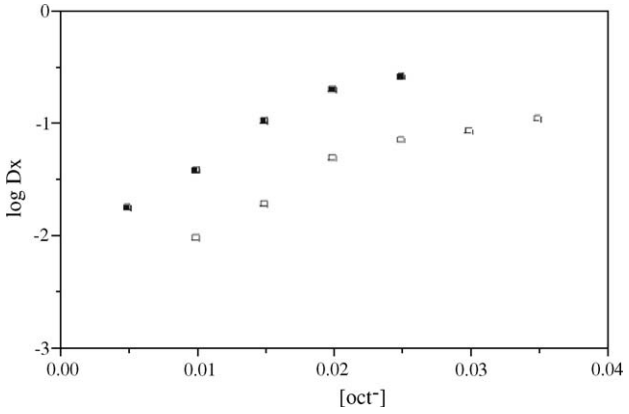


Fig. 3. Log D_x/[oct$^-$] profiles for (□) cefepime and (■) cefpirome at pH 2.0.

care was taken not to exceed the Critical Micelle Concentration. Thus, a concentration range of 10^{-2} M was selected, still below the CMC reported for octanesulphonate (Huibers et al., 1997). The [oct$^-$]/[drug] ratio was around 10^3 and the results did not depend on drug concentration. Under these conditions octanol–water partitioning experiments could be performed at pH 1.5–2.5 for cefepime and at pH 1.5–3.0 for cefpirome. Beyond this pH range the addition of octanesulphonate in the aqueous phase did not lead to measurable log D_x values under the conditions used. The effect of [oct$^-$] on log D_x followed a saturation curve in most cases. An example, including log D_x value at pH 2.0, is illustrated in Fig. 3. The linear part of the log D_x/[oct$^-$] profiles was used for the extrapolation procedure according to Eq. (3), in order to obtain log D_o, corresponding to the distribution coefficients in absence of the counter ion at the various pH values. Four to five log D_x values were used for the extrapolation procedure. The results of the extrapolation are presented in Table 2. In all cases, Eq. (3) was accompanied by a very good correlation coefficient. The slopes S were much lower than the slope generated for compound CGX-0057364, indicating a positive relation between the ion pairing effect per counter ion concentration unit and the hydrophobicity of the charged molecules.

For cefpirome direct measurements of the distribution coefficient in absence of counter ion at pH 2.5 conferred a value equal to log $D_{2.5} = -2.34$ (±0.04), slightly lower than the corresponding extrapolated log D_o value (Table 2). Practically the same value log $D_{7.4} = -2.25$ (±0.04) was obtained for the distribution coefficient of cefpirome at pH 7.4 denoting a constant lipophilicity over this pH range.

3.3. HPLC extrapolated capacity factors

Extrapolated capacity factors corresponding to pure aqueous mobile phase were determined at pH 2.5, which is close to the isoelectric points of Cef and Cefp and safe for the column. Extrapolation was linearly performed according to Eq. (4), except for Cefp when acetonitrile was used as organic modifier. In that case the quadratic relationship expressed by Eq. (5) was used (Shoenmakers et al., 1979; Bechalany et al., 1991).

$$\log k = -B\varphi + \log k_w \quad (4)$$

Table 3
Extrapolated chromatographic indexes of cefepime and cefpirome at pH 2.5

Compound	log k_w (MeOH)	r	log k_w (ACN)	r
Cefepime	1.15 (±0.04)	0.998	0.75 (±0.09)	0.994
Cefpirome	2.09 (±0.09)	0.997	4.17[a] (±0.002)	0.994

[a] Quadratically extrapolated.

$$\log k = A(\varphi)^2 - B\varphi + \log k_w \quad (5)$$

In the case of Cef the effect of organic modifier was evident and a significantly lower log k_w value was obtained with acetonitrile (Table 3). This comparison was not possible for Cefp due to the different extrapolated procedure.

4. Discussion

Calculation of partition coefficients of Cefepime and Cefpirome by ClogP and PrologPANN provided values <−2.5 (Table 1), which are difficult to be directly measured by partitioning experiments. Therefore, the ion pair concept was applied in order to enhance lipophilicity to measurable levels using different concentrations of octanesulphonate and performing linear extrapolation to log D_o values corresponding to the partitioning in absence of counter anion. To this point it should be mentioned that octanesulphonate was chosen as the counter anion after preliminary experiments with its lower homologs (hexane- and heptanesulphonate) which did not produce considerable enhancement in the apparent lipophilicity of the more hydrophilic cefepime. The procedure was first successfully applied for compound CGX-0057364, with no ionisable centres and a log P value which could be directly measured. A small effect of the counter anion was observed leading to a slightly higher extrapolated log D_o value compared to log D_{exp}. It should be noted that this difference is lower than the errors tolerated by indirect determination procedures (Lombardo et al., 2001) or calculative approaches (Mannhold et al., 1990).

In the case of the two cephalosporins application of the same approach was possible using hundredfold higher sodium octanesulphonate concentrations at pH values close to the isoelectric point of the acidic and basic function. The derived log D_o values were fairly stable at pH 2.0 and 2.5 with a tendency to decrease at pH 1.5. For Cefp measurements at pH 3.0 showed also a small decrease in log D_o. Outside the pH range 1.5–2.5 or 1.5–3.0 for Cef and Cefp, respectively, the addition of octanesulphonate did not lead to measurable log D_x values. This behavior may be explained on the basis of the underlying equilibriums. Thus, at pH < 1.5 there is only limited ionization of the carboxylic group and the zwitterionic character of the molecules is considerably reduced. Consequently the presence of two positively charged centres should produce a drastic drop in 'intrinsic' lipophilicity, which could not be overwhelmed by the ion pairing procedure. The tendency of lipophilicity to decrease at lower pH was evidenced already at pH 1.5. Another putative explanation may be the reduction in the ion pairing potential of octanesulphonate at pH <1.5 due to its own prob-

able protonation. At pH 1.5–2.5 one positively charged centre is engaged in zwitterion with the carboxylate anion, while the second positively charged centre remains available for ion pair formation. Differently, at pH ≥ 2.5 the protonation of aminothiazole is limited and octanesulphonate may compete with the carboxylate anion for the quaternary nitrogen centre, leading to the disruption of the zwitterionic structure. Thus, the inability to measure $\log D_x$ values at higher pH should rather be related to the failure of the counter anion to act as lipophilicity enhancer and not to a lower 'intrinsic' lipophilicity. In contrast, as already mentioned, the intramolecular charge compensation between the carboxylate anion and the quaternary nitrogen implies constant lipophilicity throughout a wide pH range. This was confirmed in the case of cefpirome by direct partitioning experiments at pH 2.5 and 7.4, with $\log D_{2.5} = -2.30$ and $\log D_{7.4} = -2.25$, respectively In this aspect the small decrease in the extrapolated $\log D_o$ value at pH 3.0 for cefpirome should be the result of the reduced impact of the counter anion to act as lipophilicity enhancer at this pH. The attenuation of the effect of octanesulphonate is reflected also in the significantly lower slope S of Eq. (3) (Table 2). The constant lipophilicity over a large pH range is in agreement with the constant retention of the two cephalosporins in reversed phase HPLC, previously reported (Pistos et al., 2003). In the same publication we referred also to the disruption of the zwitterionic structure as a possible explanation for the inversion of the retention/pH profile upon addition of ion pairing reagents in the mobile phase – increase in retention at low pH followed by a drastic decrease reaching a low level plateau at higher pH (Pistos et al., 2003)

Comparison of the extrapolated $\log D_o$ obtained at pH 2.5 to the experimental $\log D_{2.5}$ value, in the case of cefpirome, revealed a small positive influence of the counter ion, as observed also for the reference compound CGX-0057364. On the other hand, the extrapolated $\log k_w$ values derived by revered-phase HPLC for Cef and Cefp were significantly larger than their $\log D_o$ values. Secondary non-hydrophobic interactions (like silanophilic) may contribute to retention, which therefore cannot be considered to reflect the pure partitioning characteristics of the hydrophilic Cef and Cefp in the octanol–water system. Nevertheless, $\log k_w$ values can still be used as relative lipophilicity measures, since they provided a correct lipophilicity ranking of the two cephalosporins.

5. Conclusions

The lipophilicity of cefepime and cefpirome could be assessed applying the ion pair concept for pH values close to the isoelectric point of the acidic and basic function. In that pH range the conduction of the experiments in presence of a hydrophobic counter anion in the aqueous phase facilitated the partitioning of the two cephalosporins to octanol, circumventing the problems arising from their high hydrophilicity. This procedure could not be applied at lower pH possibly due to a further drastic decrease in the 'intrinsic' lipophilicity or to reduced ion pairing potential of octanesulphonate and at higher pH at which the ion pair formation with the quaternary charged nitrogen atom led to the disruption of the zwitterionic structure. A minor effect of the counter ion in the extrapolated $\log D_o$ values was reflected in a small positive difference compared to direct measurements in the case of Cefp and the reference compound CGX-0057364. These differences lie within the limits of acceptable errors for indirect lipophilicity determination or calculation, although further investigation could lead to a better standardization of the procedure. Thus, the method can be applied for the assessment of lipophilicity in the case of highly hydrophilic charged compounds capable to form ion pairs and is safer than the use of chromatographic techniques. However, in the case of zwitterionic species the effect of the hydrophobic ion may lead to the disruption of the intramolecular charge compensation. This issue should be considered in the investigation of the physicochemical and pharmacokinetic properties of such compounds as well as in the selection of suitable absorption enhancers.

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