ELSEVIER

www.elsevier.nl/locate/jelechem

Journal of Electroanalytical Chemistry 495 (2001) 146–151

Journal of Electroanalytical Chemistry

Voltammetric analysis of lipophilicity of benzodiazepine derivatives at the water | 1,2-dichloroethane interface

L.M.A. Monzón, L.M. Yudi *

Departamento de Físico Química, Facultad de Ciencias Químicas, Instituto de Investigaciones en Fisicoquímica de Córdoba (INFIQC), Universidad Nacional de Córdoba, Ala 1, Pabellón Argentina, Ciudad Universitaria, 5000 Córdoba, Argentina

Received 31 March 2495; received in revised form 22 September 2000; accepted 26 September 2000

Abstract

The transfer of benzodiazepine derivatives (diazepam, bromazepam, alprazolam, oxazepam, nitrazepam, clonazepam, chlordiazepoxide, flunitrazepam, midazolam and lorazepam) across the water | 1,2-dichloroethane interface was studied using cyclic voltammetry. The partition coefficients of ionic species of benzodiazepines, $\log P_{XH^+}$, were calculated from the transfer potentials measured at $pH < pK_a$. These values were compared with the partition coefficient of neutral species, $\log P_X$. The difference between $\log P_{XH^+}$ and $\log P_X$ was related to the degree of charge delocalization, which depends markedly on the presence of electron acceptor substituents in the molecule. These electron acceptor groups, in turn, affect the biological activity of these drugs. The results indicated that the difference $\Delta\log P_{XH^+} = \log P_{XH^+} - \log P_X$ can be used in structure–activity correlations as it takes into account the effects of substituents on the main positions within the molecule. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: ITIES; Ion transfer; Lipophilicity; Benzodiazepines; Drugs behavior

1. Introduction

Quantitative structure–activity relationships (QSAR) have been used extensively in the last few decades to correlate the biological activity of drugs with physicochemical parameters such as solubility, the partition coefficient in oil | water systems ($\log P$), the electronic effect of substituents, electron density, steric effects. These correlations are very important in providing a rational approach to the design of new drugs [1]. For this reason, much attention has been focused on the determination of the lipophilicity of drugs by different methods.

The interface between two immiscible electrolyte solutions (ITIES) has been used to examine kinetic and analytical aspects of the transport of various drugs [2–10], to determine partition coefficients from transfer potentials [11] and to clarify the electrochemical behavior of structurally specific and nonspecific drugs [12]. More recently, Girault and coworkers [13,14] reported ionic partition diagrams for several drugs, which define the domains of predominance of all the species present in both phases. In the same papers, these authors arrived at the important conclusion that the change in lipophilicity between charged and neutral forms of a given compound is related to the intramolecular stabilization of the charge. They showed that the nature of the substituents surrounding the charged atom as well as the degree of delocalization of the charge contribute to the stabilization of ionic species in the organic phase. Kong and Kakiuchi [15] established a lipophilicity scale for 14 monovalent arenediazonium ions and their results show the importance of charge distribution over the aromatic moiety in determining the lipophilicity.

Benzodiazepines are psychotropic drugs extensively used as hypnotics and anxiolytics [16]. Due to their high lipophilicity, they are able to partition into biological membranes and are accumulated mainly in high lipid-containing tissues [17]. Therefore, the ability of the drug to be displaced from these binding sites in membranes was employed as an alternative measure of its biological activity [18]. Fini and coworkers [19] studied the thermodynamic aspect of dissolution of benzodiazepines and reported on the relationships between the structure, the nature of the substituents, and water

* Corresponding author. Fax: +54-351-4334188.
E-mail address: mjudi@fisquim.fcq.unc.edu.ar (L.M. Yudi).

0022-0728/01/$ - see front matter © 2001 Elsevier Science B.V. All rights reserved.
PII: S0022-0728(00)00409-5

NEURELIS, INC., EX2065, page 1
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00465, U.S. PATENT 11,241,414

solubility. Biological studies lead to the conclusion that different substituents at position 7 of the benzene ring (see Fig. 1) and position 2′ of the aryl ring, produce important changes in the potency, rate of absorption and other pharmacological parameters [20,21].

In the present work, the transfer potentials of a series of benzodiazepines were determined. The aim was to correlate the difference between the partition coefficient of the neutral and charged species of these compounds with the presence of different substituents at positions 7 and 2′.

2. Experimental

The voltammetric experiments were performed in a four-electrode system using a conventional glass cell of 0.18 cm^2 interfacial area. Two platinum wires were used as counter-electrodes and the reference electrodes were Ag | AgCl. The reference electrode in contact with the organic solution was immersed in an aqueous solution of 1×10^{-2} M tetraphenyl arsonium chloride (TPAsCl) (Merck p.a.).

The base electrolyte solutions were 1×10^{-2} M NaCl (Mallinckrodt A.R.) in ultrapure water and 1×10^{-2} M tetraphenyl arsonium dicarbollyl cobaltate (TPAs DCC) in 1,2-dichloroethane (DCE, Dorwil p.a.). TPAs DCC was prepared by metathesis of tetraphenyl arsonium chloride (Sigma) and cesium dicarbollyl cobaltate (Lachema p.a.). The pH of the aqueous phase was varied in the range 0.5–11 by addition of HCl (Merck p.a.) or NaOH (Sigma).

Benzodiazepines (Clonazepam, Flunitrazepam, Chlordiazepoxide (Hoffman-La Roche), Diazepam, Alprazolam, Bromazepam (Bagó) and Nitrazepam, Oxazepam, Lorazepam, Midazolam (Monteral)) were of the highest purity available. These compounds were added to the aqueous phase in 1×10^{-3} M concentration and, depending on the pH, they can be present as neutral molecules (X), monocation (XH^+) or, in the case of midazolam, bromazepam or alprazolam, as dication (XH_2^{2+}) species.

The potential E applied between the two Ag | AgCl reference electrodes is related to the Galvani potential difference ($\Delta_o^w\phi$) across the interface by,

$$E = \Delta_o^w\phi + \Delta E_{ref}$$

where ΔE_{ref} depends on the reference electrodes and the reference solutions employed. Then, tetraethyl ammonium (TEA^+) was added to the aqueous phase, after all experiments, in order to reference all the observed standard transfer potentials, E^0_{tr,XH^+}, deduced from the peak potentials in the voltammograms. These values were then transposed to the absolute scale by applying the following relationship,

$$E^0_{tr,XH^+} - \Delta_o^w\phi^0_{tr,XH^+} = E^0_{tr,TEA^+} - \Delta_o^w\phi^0_{tr,TEA^+}$$

where $\Delta_o^w\ \phi^0_{tr,XH^+}$ and $\Delta_o^w\phi^0_{tr,TEA^+}$ are the standard transfer potentials of XH^+ and TEA^+, respectively ($\Delta_o^w\phi^0_{tr,TEA^+} = 47$ mV according to Ref. [22]).

The $\Delta_o^w\phi^0_{tr,XH^+}$ values were determined at $pH \ll pK_a$ where the current arises from the transfer of XH^+ across the interface. From these $\Delta_o^w\phi^0_{tr,XH^+}$ values, $\log P_{XH^+}$, the partition coefficient of the ionic species XH^+, is calculated according to,

$$\log P_{XH^+} = -\left(\frac{zF}{2.3RT}\right)\Delta_o^w\phi^0_{tr,XH^+}$$

These values were compared with $\log P_X$, partition coefficients of neutral species in water | 1,2-DCE, which were measured by the shake-flask method, benzodiazepine drugs were dissolved in the 1,2-dichloroethane phase containing 1×10^{-2} M TPAs DCC, while the

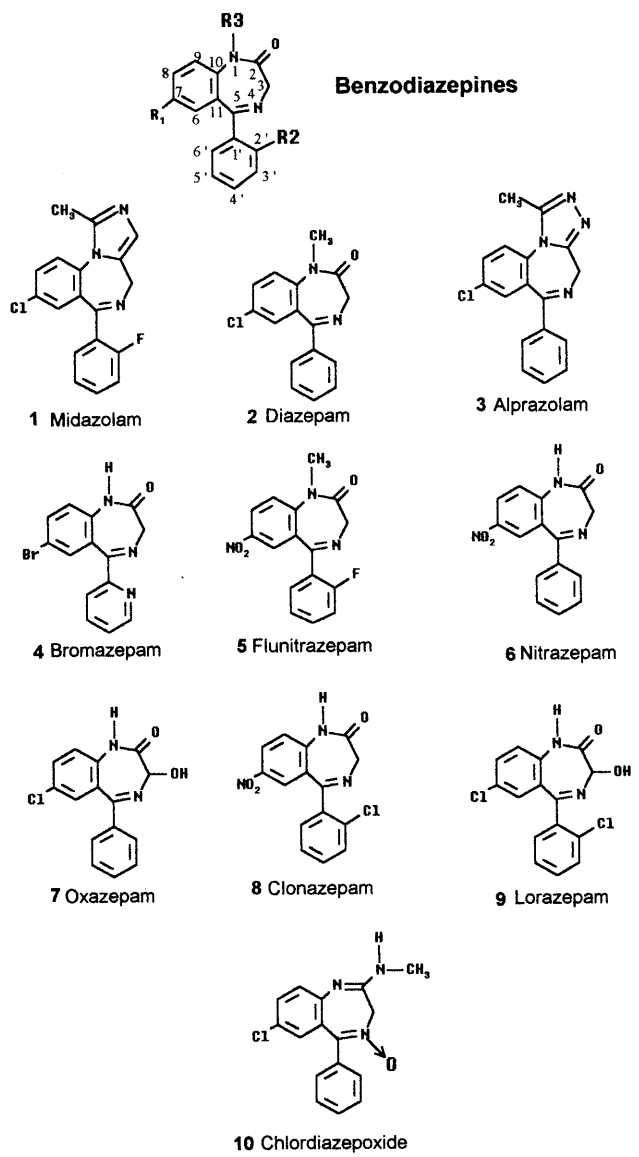

Fig. 1. Chemical structures of the benzodiazepines studied.

NEURELIS, INC., EX2065, page 2
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00465, U.S. PATENT 11,241,414

Table 1
Physicochemical parameters for benzodiazepines[a]

Compound	pK_{a_1} [b]	pK_{a_2} [b]	$\Delta_o^w\varphi^0_{tr,XH^+}$/V	$\Delta_o^w\varphi^0_{tr,XH_2^{2+}}$/V	log P_{XH^+}	log $P_{XH_2^{2+}}$	log P_X	Δlog P_{XH^+}	Δlog $P_{XH_2^{2+}}$
Midazolam	2.6	6.2	0.002	0.150	−0.034	−5.08	3.88	−3.91	−8.96
Diazepama	3.4		0.035		−0.59		3.67	−4.26	
Alprazolam	2.5		0.075		−1.27		2.89	−4.16	
Bromazepam	2.0	2.5	0.085	0.255	−1.44	-8.64	2.63	−4.07	−11.27
Flunitrazepam	1.6		0.115		−1.95		2.91	−4.86	
Nitrazepam	3.2		0.132		−2.23		3.06	−5.29	
Oxazepam	1.8		0.157		−2.66		1.90	−4.56	
Clonazepam	1.5		0.195		−3.30		3.15	−6.45	
Lorazepam	1.6		0.205		−3.47		2.09	−5.56	
Clordiazepoxide	4.8		0.135		−2.29		3.88	−6.17	

[a] They were measured or calculated as indicated in Section 2.
[b] From [19,24].

aqueous phase composition was 1×10^{-2} M NaCl, pH = 8.5. These two phases were shaken together before separation and analysis of the organic solution by UV spectroscopy.

Cyclic voltammetry was performed using a potentiostat, which eliminates the *IR* drop automatically by the means of a periodic current—interruption technique. A LyP Elect. Arg. waveform-generator and a BAS X-Y recorder (model MF-9044) were also employed. A UV-1601 Shimadzu UV spectrophotometer was used for the determination of benzodiazepine concentrations in the organic phase in the shake-flask experiments.

An AM1 semiempirical calculation [23] was used to optimize the molecular geometry of the compounds in the gaseous phase and to calculate the molecular volumes assuming a spherical model. The molecular volume of each compound was used to estimate the mean molecular radius values. Computations were conducted with the Hyperchem 5.1 package.

3. Results and discussion

The standard transfer potential of a series of benzodiazepines (Fig. 1) through the water | 1,2-DCE interface was determined by cyclic voltammetry. All the compounds have a protonation–deprotonation site on the nitrogen labeled 4 within the diazepine ring, except for chlordiazepoxide, in which protonation on this site is not allowed due to the presence of an oxygen substituent. In the latter case, a secondary amine on position 2 makes this species a monocation at pH values below pK_a. Moreover, three of the compounds analyzed (midazolam, alprazolam and bromazepam) can be present as singly (XH^+) or doubly charged (XH_2^{2+}) species depending on the pH. Table 1 shows the p*K*a values of all compounds studied, given in Refs. [19,24]. For alprazolam, the pK_a value is not available in the literature, so it was estimated from voltammetric results.

Figs. 2 and 3 show characteristic cyclic voltammograms corresponding to the transfer processes of XH^+ and XH_2^{2+} species, respectively, across the water | 1,2-DCE interface. Provided that pH $\ll$ pK_a a reversible peak system is observed for the XH^+ transfer as can be seen in Fig. 2 where the voltammogram of protonated diazepam is shown as an example. The positive peak potential, E_p^+, and the peak potential difference, $\Delta E_p = E_p^+ - E_p^- = 60$ mV, were independent

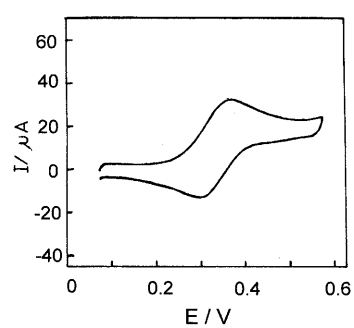

Fig. 2. Cyclic voltammogram obtained for diazepam at $v = 0.050$ V s^{-1}. Aqueous phase composition, 1×10^{-2} M NaCl + 1×10^{-3} M diazepam, pH 1.5; organic phase composition, 1×10^{-2} TPAs DCC.

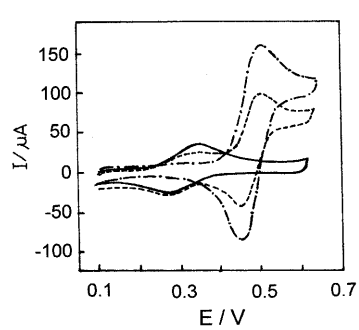

Fig. 3. Cyclic voltammogram obtained for midazolam at $v = 0.050$ V s^{-1}. Aqueous phase composition: 1×10^{-2} M NaCl + 1×10^{-3} M midazolam, pH 5.2 (–•–), 2.4 (---) and 1.4 (-•-•-); organic phase composition, 1×102^{-2} TPAs DCC.

NEURELIS, INC., EX2065, page 3
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00465, U.S. PATENT 11,241,414

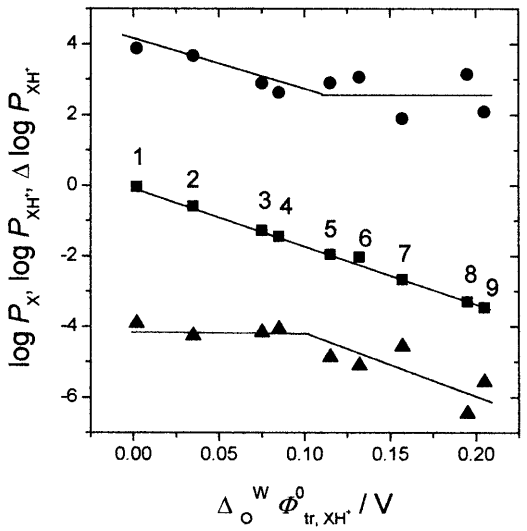

Fig. 4. Plots of (●) log P_X and (■) log P_{XH+} and (▲) Δlog P_{XH+} vs. $\Delta\phi^0_{tr,XH+}$ for XH^+ species (X = midazolam (1), diazepam (2), alprazolam (3), bromazepam (4), flunitrazepam (5), nitrazepam (6), oxazepam (7), clonazepam (8) and lorazepam (9)). Aqueous phase composition, 1×10^{-2} M NaCl 1×10^{-3} M XH^+, pH 4.0 (1), 1.0 (2, 3, 4 and 6), and 0.5 (5, 7, 8 and 9); organic phase composition: 1×10^{-2} M TPAs DCC.

of the potential sweep rate, v, and pH (for pH < pK_a). The positive peak current was proportional to $v^{1/2}$ and to the equilibrium concentration of protonated diazepam. All the XH^+ species analyzed show similar voltammetric behavior. Then, $\Delta_o^w\phi^0_{tr,XH^+}$ were calculated from $E^+_{p\,tr,XH^+}$ and $E^+_{p\,tr,TEA^+}$ values as was explained in Section 2. The results are listed in Table 1.

The voltammograms corresponding to the transfer of mono- and di-protonated species of midazolam are shown in Fig. 3. At pK_{a_1} < pH < pK_{a_2} a reversible peak system with $\Delta E_p = 60$ mV is observed due to the transfer of XH^+. As the pH is decreased, a new reversible transfer process is detected at higher potential values. In this case, $\Delta E_p = 30$ mV is in good agreement with the fact that the species transferred is the di-protonated form of midazolam, XH_2^{2+}. The peak current values obtained were proportional to the equilibrium concentration of XH^+ and XH_2^{2+} at each pH. Similar voltammetric behavior is observed for the transfer processes of both species of bromazepam. From voltammetric data, $\Delta_o^w\phi^0_{tr,XH^+}$ and $\Delta_o^w\phi^0_{tr,XH_2^{2+}}$ values were calculated as detailed above and are listed in Table 1.

No current was observed upon polarization at the interface at pH ≫ pK_a, indicating that assisted ion transfer reaction does not take place under these conditions.

Some of the benzodiazepines (bromazepam, nitrazepam, oxazepam, clonazepam and lorazepam) present an amide group at nitrogen 1 with high pK_a values (≅ 11) for deprotonation of N; however, in these cases, no current due to the anion transfer was observed at pH > 11 in the limit of the potential window.

Fig. 4 illustrates log P_{XH^+} and log P_X values versus $\Delta_o^w\phi^0_{tr,XH^+}$ obtained for all the benzodiazepines studied, except chlordiazepoxide. As can be observed, not all the compounds in the series keep the lipophilicity pattern when they are protonated with respect to neutral species. In fact, for XH^+ species with low transfer potential values (lower hydrophilicity), such as midazolam, diazepam, alprazolam, bromazepam, the log P difference between a charged and a neutral species, $\Delta\log P_{XH^+} = \log P_{XH^+} - \log P_X$, (also shown in the figure is lower than that for flunitrazepam, nitrazepam, oxazepam, clonazepam and lorazepam, transferred at higher transfer potentials (higher hydrophilicity). These results suggest that the charge effect on the hydrophilicity is not the same in all the benzodiazepines studied.

As noted by Girault and coworkers [13,14], $\Delta\log P_{XH^+}$ is a measure of the different affinity of charged and neutral forms of a compound towards an organic phase. Following Born's solvation model, they reported that the molecular volume and the intramolecular charge delocalization (i.e. the distribution of the electronic charge over more than just one atom) affect solvation of the charged species as follows,

$$\Delta\log P_{XH^+} = \frac{-(\Delta G^o_{IS} - \Delta G^w_{IS})}{RT\ln 10} = \left(\frac{z^2e^2N_A}{8\pi\varepsilon_o a}\right)\left(\frac{\varepsilon^o_r - \varepsilon^w_r}{\varepsilon^o_r\varepsilon^w_r}\right)$$

where ΔG^o_{IS} and ΔG^w_{IS} are the difference in the chemical potential between 1 mol of ions and 1 mol of neutral molecules of equal molecules of equal size in organic and aqueous phase, respectively, e, the charge of the proton; N_A, the Avogadro constant; a, the molecular radius, ε_o, the vacuum permittivity; and ε^o_r and ε^w_r are the dielectric constants of the organic and aqueous phases. Since $\varepsilon^o_r < \varepsilon^w_r$, $\Delta\log P$ is always negative. This effect is higher for ions with smaller molecular radius, ar, and large charge, z [13]. Hence, ions with a delocalized charge have $\Delta\log P_{XH^+}$ values smaller than those possessing a localized charge. This arises from the fact that the attractive forces between the charge and each solvating water molecule are weaker with a delocalized charge, which results in a smaller hydrophilicity and, thus, in a smaller change in the value of log P between neutral and protonated species.

Fig. 5 shows $\Delta\log P_{XH^+}$ values of all benzodiazepines except chlordiazepoxide and nitrazepam versus pK_a of each species, which, in turn, can be considered as a measure of charge localization in XH^+ ions (i.e. when the localization of charge is high, the XH^+ species is a strong acid, and the pK_a value is lower). As can be seen for molecules with higher pK_a, $\Delta\log P_{XH^+}$ values between −3.91 and −4.26 (alprazolam, bromazepam, diazepam, midazolam) were obtained. These values are very close to the value of −4 to −4.5 calculated for singly charged ions of molecular radius larger than 3.5 Å in water | 1,2-dichloroethane according to Born's solvation model [10,13]. In fact, the approximate molecu-

NEURELIS, INC., EX2065, page 4
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00465, U.S. PATENT 11,241,414

lar radius calculated for all benzodiazepines studied, by the AM1 semiempirical method, are between 4.0 and 4.6 Å. On the other hand, oxazepam, flunitrazepam, lorazepam and clonazepam show bigger $\Delta\log P_{XH^+}$ values (-4.56, -4.86, -5.56, -6.45). These values correspond to singly charged ions of 1.0–1.5 Å molecular radius according to Born's solvation model. There is a larger charge density in these molecules and this must be due to a lower charge delocalization. In fact, the low pK_a values for flunitrazepam, lorazepam and clonazepam indicate a big localization of charge on the nitrogen atom. Probably, the presence of electron acceptor groups NO_2, Cl and F at positions 7 and 2′, respectively, decreases the donor capacity of the non-bonding electrons pair on the nitrogen atom, decreasing the stability of XH^+ species (strong acids).

As can be noticed in Table 1, the behavior of clordiazepoxide is different from the other compounds. Its pKa value is similar to that of the first group of molecules described above, nevertheless, its $\Delta\log P_{XH^+}$ value is similar to those of the second group. Obviously, in this case, the different pK_a (4.8) value is explained taking into account the fact that the protonation site is the secondary amine on the sp^3 nitrogen at

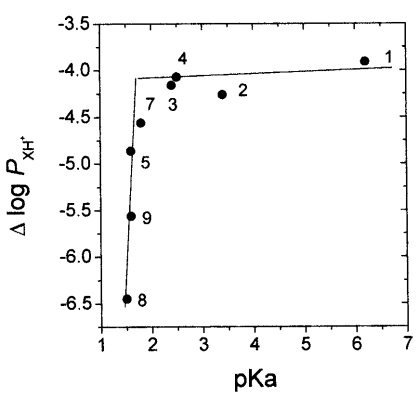

Fig. 5. Plot of $\Delta\log P_{XH^+}$ values vs. pK_a of benzodiazepines. Same experimental conditions as in Fig. 4.

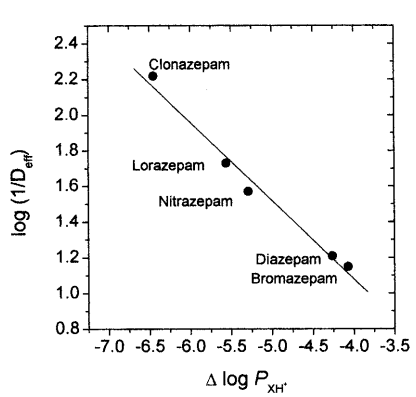

Fig. 6. Plot of biological activity vs. $\Delta\log P_{XH^+}$ for some of the benzodiazepines studied. Same experimental conditions as in Fig. 4.

position 2, which is a better electron donor than the pyridinic sp^2 nitrogen. Nevertheless, an increase of the $\Delta\log P_{XH^+}$ value is observed, probably due to the diminishing possibility of delocalization or to the fact that the charge would be better exposed to ion-dipole interactions.

In Table 1, $\Delta_o^w\phi^0_{tr,XH_2^{2+}}$, $\log P_{XH_2^{2+}}$ and $\Delta\log P_{XH_2^{2+}}$ values for XH_2^{2+} species of midazolam and bromazepam are also shown. The $\Delta\log P^{2+}_{XH_2}$ values obtained (-8.96 and -11.27, respectively) would indicate that midazolam behaves as two monocharged ions, while bromazepam can be considered a doubly charged cation, according to Ref. [13]. These differences can be explained by considering the different distances between protonatable nitrogen atoms in both molecules.

It is well known [21] that the presence of electron acceptor substituent groups at position 7 (of the benzene ring) and position 2′ (of the 5-aryl ring) increase markedly the biological activity of benzodiazepines. Fig. 6 shows a plot of $\log(1/D_{eff})$ versus $\Delta\log P_{XH^+}$. This $\log(1/D_{eff})$ parameter is used currently as a measure of pharmacological activity [12]. D_{eff} = effective dose is the drug concentration (in mg $day^{-1}\cdot$(kg of patient weight)$^{-1}$) needed to obtain the expected biological effect. The values cited in this paper were obtained from the literature [21]. A direct, linear relationship is observed. As concluded above, the presence of electron acceptor groups increases the $\Delta\log P_{XH^+}$ values as a consequence of high charge localization, and this is related directly to the biological activity. It is very interesting to note that clonazepam with an NO_2 group at position 7 and a Cl at position 2′ is the compound with higher biological activity (and higher $\Delta\log P_{XH^+}$ value) of the series analyzed. This compound is followed by lorazepam where the NO_2 group is replaced by Cl (with lower electron acceptor capacity); nitrazepam has no substituent group at position 2′ so that the activity and $\Delta\log P_{XH^+}$ values decrease. Electronegative Cl atom is present at position 7 in diazepam, but the lack of an electronegative substituent at position 2′ and the presence of a CH_3 donor group on nitrogen 1 compensates the effect and no large charge localization is observed for this molecule. A comparison of this compound with lorazepam, which has the same substituent at position 7 but also an OH group on carbon 3 and another Cl atom at position 2′ and has no CH_3 group on nitrogen 1, explains the different $\Delta\log P_{XH^+}$ values obtained for these compounds. Finally, bromazepam, without an acceptor group at position 2′ and a big size substituent as Br at position 7, is the compound with lower activity and $\Delta\log P_{XH^+}$ values. This result shows, as first demonstrated by Girault and coworkers [13], the importance of $\Delta\log P_{XH^+}$ value in quantitative structure–activity relationship studies.

NEURELIS, INC., EX2065, page 5
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00465, U.S. PATENT 11,241,414

Although benzodiazepines act as neutral molecules at physiological pH in the central nervous system, the correlation between the biological activity with $\Delta\log P_{XH^+}$ demonstrates the effect of the different substituents on the biological activity.

4. Conclusions

The determination of log P for the ionic form of a family of therapeutic compounds by transfer potential measurements at ITIES, allowed us to compare its relative hydrophobicities. Moreover, the difference between the partition coefficients of the neutral and ionized forms of each compound gives important information about the charge localization in the molecules, which depends noticeably on the molecular structure (geometry, resonance, substituent groups). This, therefore, is an important parameter to be used in QSAR relationship that takes into account not only the effect of charge in the lipophilicity of species but also the presence of electron acceptor or donor substituent groups in the molecule, which often determines its biological activity.

Acknowledgements

Financial support from Consejo de Investigaciones de la Provincia de Córdoba (CONICOR) and Secretaria de Ciencia y Técnica de la Universidad Nacional de Córdoba (SECyT) is acknowledged gratefully. The authors also thank Dr M.I. Perillo and Bago Laboratories (Argentina) for supplying kindly the benzodiazepines employed.

References

[1] C. Hansch, The use of substituent constant in structure–activity studies, in: E.J. Ariens (Ed.), Physico-Chemical Aspect of Drug Action, Pergamon Press, UK, 1968 Proceedings of the Third International Pharmacological Meeting.
[2] Y. Shao, H.H. Girault, J. Electroanal. Chem. 282 (1990) 59.
[3] Y. Shao, J.A. Campbell, H.H. Girault, J. Electroanal. Chem. 300 (1991) 415.
[4] D. Homolka, V. Marecek, Z. Samec, J. Electroanal. Chem. 163 (1984) 159.
[5] P. Vanysek, M. Behrendt, J. Electroanal. Chem. 130 (1981) 287.
[6] V. Marecek, Z. Samec, Anal. Lett. 14 (B15) (1981) 1241.
[7] F. Reymond, G. Steyaert, A. Pagliara, P.A. Carrupt, B. Testa, H.H. Girault, Helv. Chim. Acta 79 (1996) 1651.
[8] L.M. Yudi, A.M. Baruzzi, V.M. Solís, J. Electroanal. Chem. 360 (1993) 211–219.
[9] A.I. Azcurra, L.M. Yudi, A.M. Baruzzi, J. Electroanal. Chem. 461 (1999) 194.
[10] F. Reymond, Ph.D. Thesis, 1998, EPFL, Lausanne.
[11] K. Kontturi, L. Murtomaki, J. Pharm. Sci. 81 (1992) 970.
[12] K. Arai, M. Oshawa, F. Kusu, K. Takamura, Bioelectrochem. Bioenerg. 31 (1993) 65.
[13] F. Reymond, P.A. Carrupt, B. Testa, H.H. Girault, Chem. Eur. J. 5 (1999) 39.
[14] F. Reymond, V. Chopineaux-Courtois, G. Steyaert, G. Bouchard, P.A. Carrupt, B. Testa, H.H. Girault, J. Electroanal. Chem. 462 (1999) 235.
[15] Y.T. Kong, T. Kakiuchi, J. Electroanal. Chem. 483 (2000) 22.
[16] J.F. Tallman, S.M. Paul, P. Skolnick, D.W. Gallager, Science 207 (1980) 274.
[17] D.A García, M.A Perillo, Biochim. Biophys. Acta 1418 (1999) 221.
[18] I.L. Martin, Neuropharmacology 26 (1987) 957.
[19] A. Fini, A. Roda, A. Chiarini, A. Tartarini, Pharm. Acta Helv. 61 (1986) 54.
[20] J. Nelson, G. Chouinard, in: A. Baskys, G. Remington (Eds.), Brain Mechanism and Psychotropic Drugs, CRC Press, Boca Raton, USA, 1996.
[21] Goodman and Gilman (Ed.), "Las bases farmacológicas de la terapéutica", Médica Panamericans, 8va ed," (1991), México.
[22] M.H. Abraham, A.F. Danil de Namor, J. Chem. Soc. Faraday Trans. 172 (1976) 955.
[23] M.J.S. Dewar, E.G. Zoebisch, E.F. Healy, J.J.P. Stewart, J. Am. Chem. Soc. 107 (1985) 3902.
[24] K. Florey, Analytical Profiles of Drug Substances, Academic Press, San Diego, CA, 1991.

NEURELIS, INC., EX2065, page 6
PADAGIS US LLC v. NEURELIS, INC.
IPR2025-00465, U.S. PATENT 11,241,414