Physico-Chemical Profiling of Antidepressive Sertraline: Solubility, Ionisation, Lipophilicity

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Abstract: The fundamental physico-chemical parameters of sertraline, a potent selective serotonin reuptake inhibitor and reference compound in the development of new antidepressive agents, were determined. The thermodynamic solubility of the hydrochloride salt (S = 4.24 ± 0.02 mg/ml) and the free base form (S ≈ 0.002 mg/ml) was measured by the saturation shake-flask method. The co-solvent technique in methanol/water mixtures and the Yasuda-Shedlovsky extrapolation were applied for the determination of the dissociation constant (pK_a = 9.16 ± 0.02). The partition of sertraline was studied in octanol/water and alkane/water systems determining the $logP_{oct}$ and $logP_{ch}$ values by potentiometric and shake-flask methods, respectively. These experimental data were used to interpret the excellent pharmacokinetic properties of the molecule. The high lipophilicity value ($logP_{oct}$ = 4.30 ± 0.01) of the nonionised form confirms the good absorption and distribution in the body. However, the good brain penetration can better be explained with the lack of polar interactions evidenced here by the zero ΔlogP ($logP_{oct} - logP_{ch}$) value of sertraline.

Key Words: Sertraline, physico-chemical profiling, pharmacokinetics, logS, pK_a, logP.

INTRODUCTION

Physico-chemical profiling is a fundamental tool at the early stage of drug discovery in screening drug-like candidates. The new strategy in drug research prefers the parallel evaluation of efficacy and pharmacokinetic properties [1]. Many efforts have been made to predict the ADME (absorption, distribution, metabolism, excretion) features using physico-chemical parameters. For decades, lipophilicity in terms of octanol/water partition coefficient (logP) was used as a single parameter for this purpose in drug design. Later on, the protonation/dissociation ($logK/pK_a$) constants were recognised as parameters that set the ionisation state of the molecule in various compartments of the body. Recently, complex physico-chemical profiling, including molecular properties such as solubility, hydrogen bonding and permeability, has been found to be of predictive power in ADME [2].

In the present work we carried out the complex physico-chemical profiling of sertraline, a potent antidepressive drug, frequently used as a reference compound in the development of new antidepressive agents [3, 4].

Sertraline – (1*S*,4*S*)-4-(3,4dichlorophenyl)-1,2,3,4-tetrahydro-N-methyl-1-naphthalenamine – belongs to the selective serotonin reuptake inhibitors (SSRI). It is the second most potent inhibitor of 5-HT reuptake and the second most selective blocker of serotonin over norepinephrine reuptake [5]. Due to its favourable side effect profile, safety in overdose and tolerability, sertraline is widely used in depression, anxiety disorders and social phobia [6]. It was therefore sold among the top ten pharmaceutical products in 2003 [7].

Sertraline has good pharmacokinetic properties. It is administered orally usually in a daily dose of 50-200 mg [8]. Its absorption from the gastrointestinal tract is almost complete and 8-10 hours are needed to reach the maximum plasma concentration [9]. Sertraline binds to the plasma proteins and has a large volume of distribution [10]. It has excellent brain penetration property. The brain concentration in rats has been found 40 times higher than in plasma [11]. The main elimination pathway is hepatic metabolism *via* N-demethylation [12]. N-desmethylsertraline has only 5-10 % of the serotonin reuptake inhibitor potency relative to the parent molecule [13]. Sex and age-dependent differences have also been shown in tissue distribution and metabolism [14].

A number of chromatographic methods have been reported for the determination of sertraline in biological samples [15, 16] and UV spectroscopy was used for quality control in pharmaceutical formulations [17, 18].

Only a few physico-chemical parameters of sertraline have been reported in the literature. Johnson and Chang [19] determined the apparent dissociation constant (p_sK_a) in ethanol/water 50:50 v/v% and in methanol/water 40:60 v/v% and found 8.5 and 8.6 values, respectively. The aqueous pK_a value (9.48±0.04) was calculated from potentiometric titration in water using an obsolete and non-validated approximation method. The aqueous solubility data of a saturated solution of sertraline hydrochloride at different pH values have been published but without statistical parameters. Similar data for sertraline (L)-lactate have been reported [20]. No data about the lipophilicity of the molecule has been published.

Here we report the most important physico-chemical parameters of sertraline, namely the thermodynamic solubility

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in water (logS), the aqueous dissociation constant (pK_a) and the partition coefficient (logP) in two solvents systems (octanol/water and cyclohexane/water) determined by validated methods. Then, these highly precise experimental data are used to find a better insight into the pharmacokinetic processes of sertraline and they may serve as reference data in the development of new antidepressants.

MATERIALS AND METHODS

Materials

Sertraline hydrochloride was generously supplied by Gedeon Richter Ltd. (Budapest) and used without further purification. Octanol was of HPLC grade (Aldrich), distilled water was of pharmacopoeial grade (Ph. Eur. 4.) and all other reagents were of analytical grade. The Britton-Robinson buffer (acetic, phosphoric and boric acids, each at 0.04 M, treated with 0.2 M NaOH) was used as the aqueous phase of pH 7.4 and 8 in lipophilicity measurements and at 11.5 in the solubility study.

Apparatus

For the potentiometric titrations PCA 101 and GLpKa automated analysers (Sirius Analytical Instr. Ltd. Forest Row, UK), for the spectrophotometric measurements Jasco V-550 spectrometer (Easton, USA), for pH measurements Radiometer MeterLab PHM200 pH meter (Lyon, France), for separation of partitioning phases Hermle Z 382 K centrifuge (Wehingen, Germany) and for shaking Lauda M20S thermostated shaker (Lauda-Königshofen, Germany) were used.

Determination of the Thermodynamic Solubility

The thermodynamic solubility was measured by the standard saturation shake-flask method [21, 22].

The thermodynamic solubility of the hydrochloride salt of sertraline was determined in distilled water. Solubility of the nonionised (basic) form in Britton-Robinson buffer at pH 11.5 was also investigated. The saturated solution containing solid excess of the sample was stirred for 48 hours at a temperature of 25.0 ± 0.1 °C allowing to reach the thermodynamic equilibrium. After a further 24 hours of sedimentation, the concentration in the supernatant was measured by UV spectroscopy. Two parallel solubility measurements were performed, three aliquots were analyzed from each, and the results were calculated using six data points.

The specific absorptivity ($A^{1\%}_{1cm}$) was determined in distilled water, at the selected wavelength (λ_{max}=273 nm), using 16 points of two parallel dilution series. The data were obtained from the linear regression equation of the Lambert-Beer law.

Determination of the Dissociation Constant

Due to the poor water solubility of sertraline the dissociation constant (pK_a) was determined by mixed solvent procedure [23, 24].

The electrode standardisation was performed by the four-parameter procedure in semi-aqueous medium. HCl solutions of known concentration, containing 43.64-63.97 wt % methanol were titrated with standardised KOH at 25.0 ± 0.1 °C, at I = 0.15 M ionic strength using KCl, under nitrogen atmosphere, in the pH interval 1.8 – 12.2. The operational pH reading was related to the p_cH values by the standard multiparametric equation:

$$pH = \alpha + Sp_cH + j_H[H^+] + j_{OH}K_w/[H^+] \quad (1)$$

The parameters were determined by a weighted non-linear squares procedure.

10 ml of 0.992-1.045 mM semi-aqueous solutions of the samples containing 43.64-63.97 wt % methanol were pre-acidified to pH 3 with 0.5 M HCl, and were then titrated with 0.5 M KOH to pH 12. The titrations were performed at 25.0 ± 0.1 °C, under nitrogen atmosphere, at I = 0.15 M ionic strength using KCl. Measurements were carried out in three different methanol/water mixtures. Titrations in each mixture were repeated twice. The apparent ionisation constants in the mixed solvent (p_sK_a) were calculated from the difference (Bjerrum) plot. The Yasuda-Shedlovsky procedure was applied to estimate the aqueous pK_a value.

Determination of the Partition Coefficient in the Octanol/Water System

The logarithm of octanol/water partition coefficient ($logP_{oct}$) was determined by the dual-phase potentiometric titration [25, 26].

8 ml of 0.93-1.05 mM aqueous solutions of samples were titrated under the same conditions as in pK_a determinations but with the presence of a partitioning solvent, water-saturated octanol. The following phase ratios were applied: 8.0 ml water : 0.1 ml octanol and 8.0 ml water : 0.15 ml octanol. From the octanol containing titrations the apparent ionisation constants (p_oK_a) and the logP value were calculated using the equation below:

$$P = (10^{(pKa-poKa)}-1) / r \quad (2)$$

where r is the volume ratio of the organic and aqueous phases. Six parallel titrations were carried out.

Determination of the Partition Coefficient in the Cyclohexane/Water System

The partition coefficient in the cyclohexane/water system ($logP_{ch}$) was determined by the traditional shake-flask method [27, 28, 29].

Before measurements, the aqueous and organic phases were saturated with each other by shaking. The phases were then allowed to separate on standing and they were then filtered. Britton-Robinson buffer solutions of pH 7.4 and 8 were used as the aqueous phase. The following phase ratios were used: 10 ml water : 0.1 ml cyclohexane and 20 ml water : 0.1 ml cyclohexane at pH 7.4 and 30 ml water : 0.1 ml cyclohexane at pH 8. The sample was dissolved in the aqueous phase, the concentration was measured by spectrophotometry at λ_{max}= 273 nm before the partition, then cyclohexane was added to the solution and the phases were equilibrated by shaking for 1 hour, at 25.0 ± 0.1 °C. After separation of the phases in a centrifuge at 2000 rpm for 10 min, the concentration of the solute was determined in the aqueous phase again (after partition). From the concentration

decrease the $logP_{ch}$ was calculated. Six parallel measurements were performed at each pH.

RESULTS AND DISCUSSION

Sertraline (Fig. **1**) is a tetrahydro-naphthalenamine derivate, containing an aliphatic secondary amino group as basic site. Poor water solubility and high lipophilicity of the nonionised form can be expected, due to the one single polar functional group in the molecule.

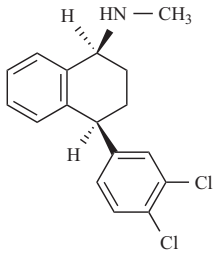

Fig. (**1**). The structure of sertraline.

Solubility

The thermodynamic solubility of sertraline was determined by the traditional shake-flask method. Solubility of the hydrochloride salt (BHCl) was measured in distilled water at pH 6.05, the pH of the saturated solution of the salt. The experimentally obtained spectroscopic data ($A^{1\%}_{1cm}$ and statistical parameters) and solubility values are listed in Table **1**. We also tried to determine the solubility of the nonionised (B) form at pH 11.5, where this species predominates by more than 99.5 %. Because of the poor solubility of sertraline base, the specific absorptivity ($A^{1\%}_{1cm}$) at this high pH could not be measured. We have found however, that the UV spectrum of the molecule is not pH dependent (as the protonating nitrogen and the nearest aromatic ring are separated by two σ-bonds), and therefore the $A^{1\%}_{1cm}$ value determined at pH 6.05 could be used for the calculation of the solubility of the base, as a good approximation.

Data in Table **1** show the rather poor water-solubility of sertraline. The hydrochloric salt is soluble in 0.424 %, which corresponds to the "*slightly soluble*" category in European Pharmacopoeia. As expected, the free base form has more than 3 orders of magnitude lower solubility in water. Obviously, the sufficient dissolution of the salt form from tablets (50 mg dose) is assured by the ~ 200-500 ml volume of the gastric fluid.

Ionisation

Sertraline has one proton-binding site, the ionisation ability of the molecule can be characterised by the dissociation constant (pK_a).

Generally, potentiometry in aqueous medium is the method of choice for the pK_a determination for molecules of solubility higher than 0.5 mM concentration in the whole pH interval of the titration [30]. As we have seen above, the solubility of the nonionised (B) form of sertraline is around 0.002 mM and thus it precipitates during the titration at higher pH values. Therefore, the "*co-solvent method*" was applied for the pK_a determination using methanol/water mixtures. We followed all recommendations previously suggested in a validation study of this method [24]. The p_sK_a values were measured in three different methanol/water mixtures and the pK_a value was obtained by extrapolation to zero methanol content. Table **2** summarises the results obtained in 6 separate titrations in mixtures of 43.65 – 63.97 % methanol, and Fig. (**2**) shows the Yasuda-Shedlovsky plot. Concerning the structure of sertraline the extrapolated aqueous 9.16 pK_a is a reasonable value.

Using the pK_a value, the percentage concentration of the neutral and cationic sertraline species in the pH range of 2 – 12 were calculated. Distribution curves (Fig. **3**) show that sertraline is present mainly in the ionised (BH^+) form in different compartments of the human body (at stomach pH of 1.5: 100 %; at gastrointestinal tract average pH of 6.4: 99.83 %; at tissue pH of 7.4: 98.29 %).

The high predominance of the ionised, polar form is not favourable for the transport. However, numerous studies indicate that the dielectric constant (ε) in the region close to the phospholipid bilayer is ~ 32 (the same as the value of methanol) [2]. Using our experimental p_sK_a value obtained in 64 wt % MeOH (ε=49.5) the presence of BH^+ decreases to 94.19 % at tissue pH. The p_sK_a value valid in pure methanol (approximate pK_a^{memb}) was calculated from the Yasuda-Shedlovsky equation (Table **2**) and has been found to be 8.2. Using this value the calculated ratio of BH^+ *vs* B is 86.32 % *vs* 13.68 %, which shows considerable increase of the transport (B) form.

Lipophilicity

The lipophilicity of sertraline is characterised here by the logarithm of partition coefficient (logP) values obtained in two solvent systems. The $logP_{oct}$ was measured in the octanol/water system using the dual phase potentiometric

Table 1. The Spectroscopic and Thermodynamic Solubility Data of Sertraline

Ionisation form of sertraline	Spectroscopic data				Solubility		
	λ_{max}	$A^{1\%}_{1cm}$	r	n	mg/ml±SD	M	logS
BH^+ (in distilled water, pH=6.05)	273	28.5	0.9997	16	4.24 ± 0.02	$1.24\ 10^{-2}$	-1.91
B (at pH=11.5)	cannot be measured				0.002	$5.9\ 10^{-6}$	-5.23

Table 2. Apparent Dissociation Constants (p_sK_a) in Methanol/Water Mixtures and Aqueous Dissociation Constant (pK_a) Obtained by Yasuda-Shedlovsky Extrapolation

wt% methanol	[H_2O] (mol/l)	_	p_sK_a	SD	n
43.65	28	58.9	8.85	0.01	2
53.57	22.5	54.5	8.73	0.01	2
63.97	17.1	49.5	8.61	0.02	2
$p_sK_a + \log[H_2O] = 12.783 - 146.5 / \_ \; r^2 = 0.9934$					
$pK_a = 9.16 \pm 0.02$					

titration. This method, as the "*gold standard*" of logP determination [2] provides a powerful technique for logP measurements even for compounds of high lipophilicity, being fast, precise and automated. The $logP_{oct}$ value of sertraline was calculated from six potentiometric titrations using two different phase-ratios (80 and 53.3) and has been found to be 4.30 ± 0.01 (Table **3**). Fig. (**4**) exhibits the lipophilicity-pH profile of the molecule.

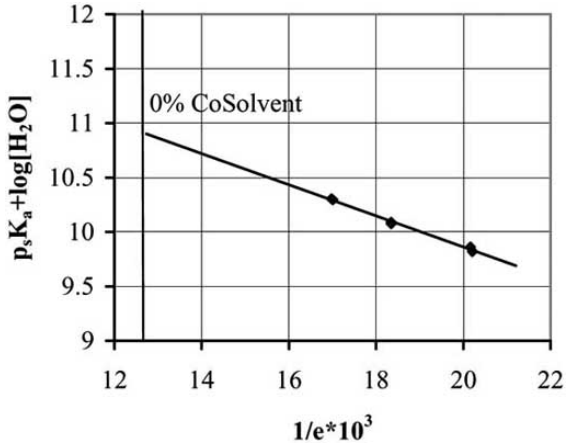

Fig. (**2**). Yasuda-Shedlovsky plot.

Albeit pH-metry (using Sirius instruments) is useful also in case of solvent systems other than octanol/water (e.g. 1,2 dichloroethane, chloroform, etc,), we have not found it ideal for the cyclohexane/water system, due to the high volatility of the organic solvent. Thus, the $logP_{ch}$ of sertraline was determined by the traditional shake-flask method. The distribution coefficients ($logD^{pH}_{ch}$) were measured at two pH values (7.4 and 8) and the true $logP_{ch}$ (referring to the nonionised form) was calculated based on the following equation:

$$\log P = \log D + \log (1+10^{pKa-pH}) \quad (3)$$

Data in Table **3** show interesting partition behaviour of sertraline, since the logP values are identical in the two different solvent systems. While octanol is an amphiprotic solvent, capable of both H-bond donor and acceptor interactions with the solute, the alkane- type cyclohexane is considered to be inert (no polar interactions, the solvation is based on the van der Waals effect). The identical logP values of sertraline mean that the molecule has no propensity for H-bond formation. Rather, the main solvation interaction in the partition process is the dispersion effect with the organic solvent.

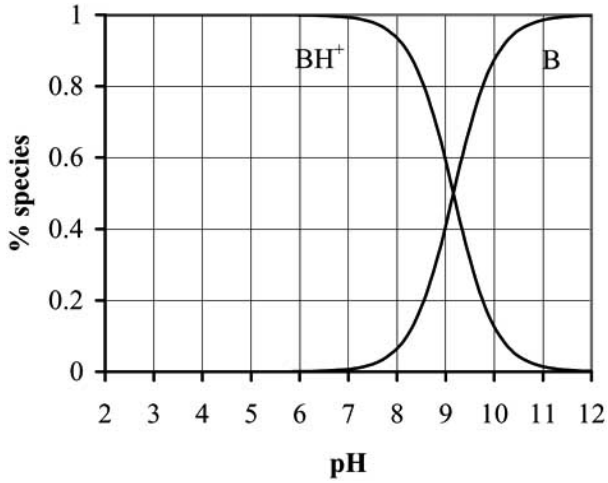

Fig. (**3**). Distribution curves of the species.

The logP data in two solvent systems explain the unusual pharmacokinetic properties of sertraline. The high $logP_{oct}$ value of the nonionised form predicts the good absorption and fast distribution in the body, however it does not completely models alone the high brain penetration.

The ΔlogP value, defined as the difference of $logP_{oct}$ and $logP_{ch}$, is a better measure of partition from blood to brain (logBB), where the governing molecular step is donating of H-bonds to the hydrophilic parts of lipids in the blood-brain barrier [31, 32, 33, 34]. ΔlogP=0 value of sertraline indicates the lack of H-bond formation and explains well its high brain concentration.

Table 3. LogP Values of Sertraline in Octanol/Water and Cyclohexane/Water Systems

solvent system	logP ± SD (n)	method
octanol/water	4.30±0.01 (6)	pH-metry
cyclohexane/water	4.30±0.12 (12)	shake-flask

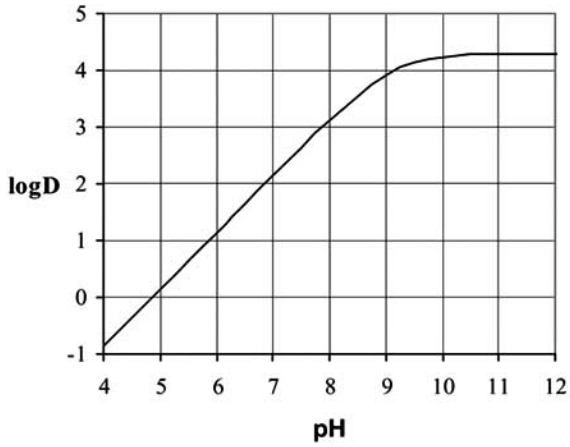

Fig. (**4**). The apparent (non-specific) lipophilicity – pH profile of sertraline in the octanol/water system.

CONCLUSIONS

In this study the most important physico-chemical parameters of sertraline have been determined. The thermodynamic solubility of its hydrochloride salt was found to be 4.24 mg/ml, while the intrinsic solubility of the nonionised form is lower by more than 3 orders of magnitude. This is in line with the high lipophilicity of the molecule ($logP_{oct}$ = 4.30). Based on the partitioning experiments in two solvent systems, sertraline is not capable for H-bond formation as evidenced by the ΔlogP=0 value.

The complex physico-chemical profiling allowed to interpret the excellent pharmacokinetics of the molecule. The solubility assures sufficient dissolution from the solid dosage form in the gastric fluid, where the molecule is overwhelmingly present in its protonated form. The almost complete absorption is governed by the high lipophilicity of sertraline base *via* transcellular route through the membranes. At the pH of the plasma, the 98.29 % dominance of the BH^+ form favours the binding to proteins and thus the distribution in the body. However, the good brain penetration (and the high logBB coefficient) can be explained with the lack of polar interactions (H-bond formation) indicated by the zero ΔlogP value.

This study provides a better insight into the relation between physico-chemical properties and pharmacokinetic data of sertraline and can be used as basic information in the development of new antidepressive drugs.

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