ELSEVIER

Journal of Pharmaceutical and Biomedical Analysis
30 (2002) 1087–1092

JOURNAL OF
PHARMACEUTICAL
AND BIOMEDICAL
ANALYSIS

www.elsevier.com/locate/jpba

^{19}F NMR spectrometric determination of the partition coefficients of some fluorinated psychotropic drugs between phosphatidylcholine bilayer vesicles and water

Ahmed A. Omran [a], Keisuke Kitamura [a,*], Shigehiko Takegami [a], Manabu Kume [a], Megumi Yoshida [a], Abdel-Aziz Y. El-Sayed [b], Mahmoud H. Mohamed [c], Mohamed Abdel-Mottaleb [b]

[a] *Kyoto Pharmaceutical University, 5 Nakauchicho, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan*
[b] *Faculty of Science, Department of Chemistry, Al-Azhar University (Assiut Branch), Assiut 71511, Egypt*
[c] *Faculty of Pharmacy, Department of Pharmacognosy, Al-Azhar University (Assiut Branch), Assiut 71511, Egypt*

Received 16 February 2002; received in revised form 27 May 2002; accepted 27 June 2002

Abstract

A simple ^{19}F NMR spectrometric method was proposed for the determination of the partition coefficients of fluorinated psychotropic drugs, trifluoperazine (TFPZ), flunitrazepam (FNZ) and flurazepam (FZ) between phosphatidylcholine (PC) bilayer of small unilamellar vesicles (SUVs) and water (buffer). Each ^{19}F NMR spectrum of these drugs in the presence of PC SUV showed a single signal accompanying a PC concentration-depending shift change and broadening, which indicated a fast exchange of these drugs between the water phase and the PC bilayer of SUV. From the relationship between the ^{19}F chemical shift change ($\Delta\delta$) of each drug and the PC concentration, the molar partition coefficients (K_p's) were calculated and obtained with a good precision of RSD below 6%. The fractions of the partitioned drugs calculated by using the obtained K_p-values were in a good agreement with the experimental values. The results demonstrate that the ^{19}F NMR method can be usefully applied to the determination of partition coefficients of many drugs having fluorine atom(s) without any separation procedure, especially for drugs which do not have absorption in the ultraviolet or visible region, or those having absorption but show insignificant spectral changes according to their incorporation to PC bilayers (e.g. FNZ).

Keywords: 19F NMR; Partition coefficient; Liposome; Trifluoperazine; Flunitrazepam; Flurazepam

1. Introduction

It is becoming increasingly recognized that the partition coefficients of drugs obtained with liposome (phospholipid bilayer vesicles)/buffer systems are more useful than that derived with the

* Corresponding author. Tel.: +81-75-595-4659; fax: +81-75-595-4760
E-mail address: kitamura@mb.kyoto-phu.ac.jp (K. Kitamura).

0731-7085/02/$ - see front matter
PII: S0731-7085(02)00438-7

traditional octanol/water system in the structure–activity relationship studies of drugs [1–3]. Also, the partition coefficients of drugs between phospholipid bilayer vesicles and water are important in the investigation of the drug interactions with biomembranes [4–7].

Among the widely used modern drugs, there are many important drugs which contain fluorine atom(s). To determine the partition coefficients of these drugs in the liposome/buffer systems, ^{19}F NMR spectrometry [8–10] will be highly useful since it has a large chemical shift range with high sensitivity (90% of proton) and therefore it is easy to measure the chemical shift changes induced by the interaction with phospholipid bilayer membranes. Furthermore, as ^{19}F nucleus is not contained in natural biological substances, ^{19}F NMR signals of the fluorinated drugs can be measured without interference of the background signals.

In this paper, we attempted to develop a simple ^{19}F NMR spectrometric method to determine the partition coefficients of fluorinated psychotropic drugs, trifluoperazine (TFPZ), flurazepam (FZ) and flunitrazepam (FNZ) for a phosphatidylcholine small unilamellar vesicle (PC SUV)/buffer system. The chemical structures of these drugs are shown in Fig. 1.

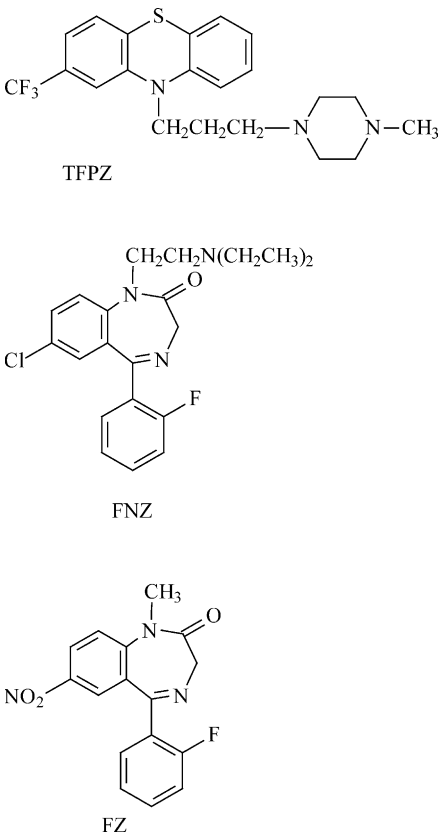

Fig. 1. The chemical structures of fluorinated psychotropic drugs studied.

2. Experimental

2.1. Calculation method

The molar partition coefficient of a drug between PC bilayer of SUV and water is defined as [6]

$$K_p = \frac{([B_L]/[B_T])/[L]}{([B_W]/[B_T])/[W]} \quad (1)$$

where $[B_L]$ and $[B_W]$ represent the concentrations (the number of moles per liter of the sample solution) of the drug incorporated in the PC bilayer and the free drug in the water phase, respectively, and $[B_T]$ equals to the concentration of the total amount of the drug added to the sample solution ($[B_T]=[B_L]+[B_W]$). $[L]$ and $[W]$ are molar concentrations of PC and water (55.4 M at 21 °C), respectively.

Eq. (1) is equivalent to

$$K_p = \frac{B_L/[L]}{B_W/[W]} \quad (2)$$

where B_L and B_W represent the fractions of the drug in the PC bilayer and water, respectively.

Since the magnetic environment of the drug incorporated in PC bilayer is different from that of free drug in water phase, the chemical shift of the ^{19}F signal of the partitioned drug will be different from that of the free drug in water phase. Then we express the ^{19}F chemical shift of the drug in the lipid phase as δ_L and that in the water phase as δ_W. If the exchange rate of the drug between lipid and water phases is fast enough at the time scale of ^{19}F NMR, the observed chemical shift (δ_{obs}) of the

^{19}F signal can be expressed as

$$\delta_{obs} = B_L\delta_L + B_W\delta_W \quad (3)$$

A new variable is introduced to represent the difference between δ_{obs} and δ_W as

$$\Delta\delta = \delta_{obs} - \delta_W = B_L(\delta_L - \delta_W) \quad (4)$$

As the value of $(\delta_L - \delta_W)$ is constant, $\Delta\delta$, the chemical shift difference of the drug before and after the addition of PC SUV, is proportional to the fraction of the drug in the PC bilayer, B_L. Thus, B_L can be shown as

$$B_L = \frac{\Delta\delta}{\Delta\delta_{max}} \quad (5)$$

where $\Delta\delta_{max} = \delta_L - \delta_W$.

Using Eq. (5) and $B_W = 1 - B_L$, Eq. (2) can be rewritten as follows:

$$K_p = \frac{(\Delta\delta/\Delta\delta_{max})/[L]}{(1 - (\Delta\delta/\Delta\delta_{max}))/[W]} \quad (6)$$

which can be rearranged to

$$\Delta\delta = \frac{K_p\,\Delta\delta_{max}[L]}{K_p[L] + [W]} \quad (7)$$

The values of K_p and $\Delta\delta_{max}$ can be calculated from the experimental values of $\Delta\delta$ and $[L]$ by applying a nonlinear least-squares method (accompanying a Taylor expansion) to Eq. (7) [6].

2.2. Reagents

Trifluoperazine (TFPZ) dihydrochloride, flunitrazepam (FNZ) and flurazepam (FZ) dihydrochloride were purchased from Sigma (St. Louis, MO) and used without further purification but good purity was confirmed by ^1H NMR, thin layer chromatography (TLC) and melting point measurements. Egg yolk L-α-phosphatidylcholine (PC, purity > 99%, 20 mg/ml chloroform solution) was obtained from Avanti Polar Lipids, Inc. (Alabaster, AL) and its purity was confirmed by TLC. Other chemicals were of analytical reagent grade.

2.3. PC SUV preparation

An appropriate volume of the PC stock solution was evaporated by using a rotary evaporator. The chloroform residue was further removed by applying a high vacuum at room temperature for more than 4 h. Thereafter, 5 ml of buffer was added and vortexed to make a homogeneous PC suspension. The buffer used was HEPES buffer (50 mM NaCl, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, pH 7.4) or the same one but contains 10% D_2O. The suspension was subsequently sonicated into SUV by an ultrasonicator UD-200 (Tomy Seiko Co. Ltd., Tokyo, Japan) at a power level 5.0 under a gentle stream of nitrogen in an ice bath. Net sonication time of 30 min was applied by repeating 10 consecutive cycles of 3 min sonication with 3 min interval. The sonicated suspension was centrifuged for 20 min at 3500 rpm ($2000 \times g$) to eliminate sediment from the sonication tip. The exact PC concentration in the sonicated suspensions was calculated by phosphorus determination [11]. The size distribution of PC SUV was confirmed by a submicron particle analyzer (Nicomp Model 380, Santa Barbara, CA) as previously reported [7].

2.4. ^{19}F NMR experiments

To each of several 2 ml volumetric flasks containing a suitable amount of FNZ or FZ or TFPZ stock solution, a suitable aliquot of the PC SUV suspension was added, and the buffer was further added to volume. The final concentrations of TFPZ, FNZ and FZ were 100, 100 and 200 μM, respectively. After shaking each flask for a short time, ca. 1 ml of the sample solution was transferred to a 5 mm diameter NMR tube. A coaxial internal tube containing ca. 5 mM trifluorotoluene in $CHCl_3$ (for TFPZ) or sodium 2-fluorobenzoate (for FZ and FNZ) in D_2O was carefully inserted to the sample tube to provide a reference signal. The sample tube was then capped tightly and sealed with paraffin film. These reference compounds were employed to ensure the precision in the chemical shift measurement, since they have similar resonance frequencies to that of the drugs to which they were adopted. The deuterium lock

signal in the experiment of TFPZ was provided by D_2O in the buffer (ca. 10%), and in the experiments of FZ and FNZ by the D_2O solvent in the coaxial internal tube. ^{19}F NMR spectra were measured by using a Varian ^{unity}Inova spectrometer operating at 376.21 MHz without proton decoupling. The probe temperature was 21 ± 2 °C. The number of FID accumulations to improve the signal-to-noise ratio was 2000–12 000.

2.5. UV absorption and second-derivative spectra

The UV sample solutions containing 100 μM TFPZ or 200 μM FZ and various amounts of PC SUV were prepared in a similar manner to the NMR sample preparation. The reference solutions were those prepared without the drug. The absorption spectra were measured by using a double-beam spectrophotometer (HITACHI U-3210). The spectrophotometer was controlled by a personal computer (NEC PC-9801) through an RS-232C interface and the spectral data were stored in the personal computer. The absorption spectra were measured at 21 ± 2 °C using 1 mm light-pass length matched quartz cuvettes with a slit width of 2 nm and wavelength interval of 0.1 nm. The second-derivative spectra were calculated in the same way as previously reported [6,12].

3. Results and discussion

3.1. ^{19}F NMR spectra

The ^{19}F NMR spectra of FNZ in the sample solutions containing various amounts of PC SUV were depicted in Fig. 2 as a typical example. FNZ shows a downfield shift and signal broadening depending on the increase in the PC SUV concentration due to its interaction with the PC bilayer. FNZ shows a single signal at any PC concentration studied, which proves that the exchanging rate of FNZ between the free and partitioned states is fast enough as compared to the ^{19}F NMR time scale. TFPZ and FZ showed similar results except the direction of the induced shift of TFPZ was upfield.

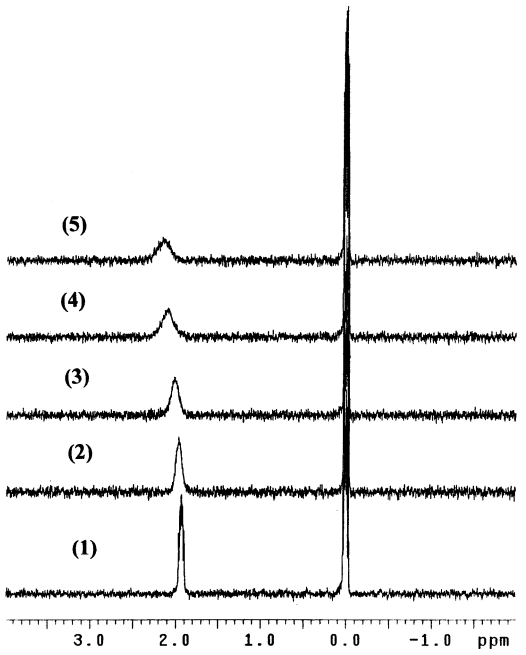

Fig. 2. ^{19}F NMR spectra of 100 μM FNZ in HEPES buffer solutions (pH 7.4, 21 ± 2 °C) containing various amounts of PC SUV. The reference is sodium 2-fluorobenzoate. PC SUV: (1) 0 mM; (2) 0.10 mM; (3) 0.20 mM; (4) 0.40 mM; (5) 0.50 mM.

3.2. Calculation of K_p and $\Delta\delta_{max}$

The chemical shift (δ_{obs}) of FNZ for each spectrum in Fig. 2 was measured and the $\Delta\delta$-values for FNZ were given by the differences of the δ_{obs}-values between spectrum (1) and spectra (2)–(5) in Fig. 2, respectively. Using these $\Delta\delta$-values and the PC SUV concentrations, the K_p- and $\Delta\delta_{max}$-values of FNZ are calculated by the non-linear least-squares method. By the same manner,

Table 1
K_p- and $\Delta\delta_{max}$-values of the fluorinated psychotropic drugs between PC SUV and water

	K_p ($\times 10^{-3}$)[a]	$\Delta\delta_{max}$ (ppm)[a,b]
TFPZ	227 ± 12 (217 ± 8)[c]	-0.194 ± 0.005
FNZ	12.5 ± 0.7	1.685 ± 0.079
FZ	8.9 ± 0.3 (9.6 ± 0.5)[c]	1.466 ± 0.010

[a] Mean ± S.D. ($n = 3$).
[b] Negative value indicates an upfield shift.
[c] Measured by second-derivative spectrophotometry.

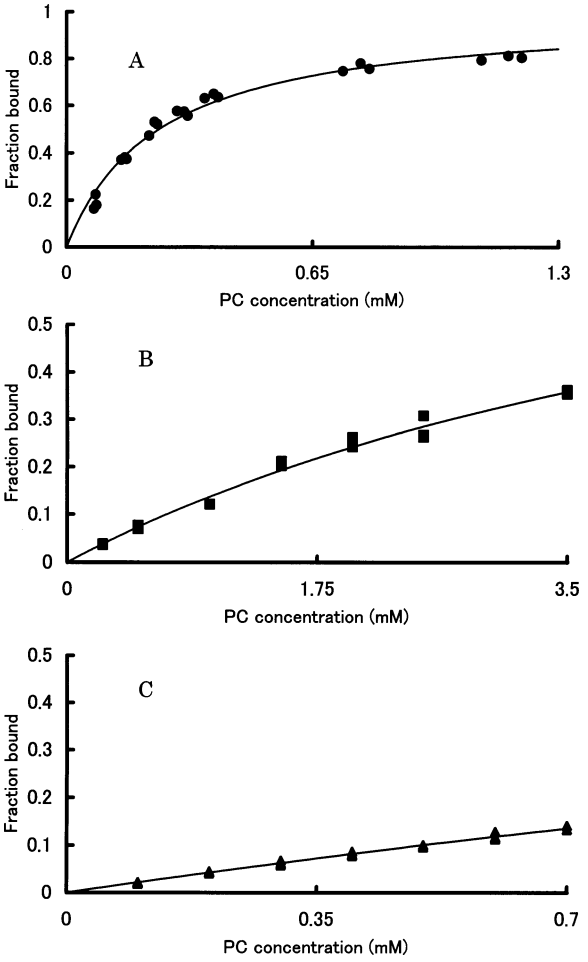

Fig. 3. Fraction ($\Delta\delta/\Delta\delta_{max}$) of (A) TFPZ, (B) FNZ and (C) FZ in PC SUV bilayer at various PC SUV concentrations. The solid lines show the theoretical curves calculated from Eq. (7) using the obtained K_p- and $\Delta\delta_{max}$-values. The symbols are the experimental values (mean ± S.D.): (●) TFPZ, (■) FNZ, and (▲) FZ.

the K_p- and $\Delta\delta_{max}$-values of TFPZ and FZ were calculated. The obtained K_p- and $\Delta\delta_{max}$-values are listed in Table 1 and the results show a good reproducibility with the relative standard deviations below 6%.

To confirm the accuracy of K_p-values obtained by ^{19}F NMR measurements, the K_p-values of TFPZ and FZ were determined by the second-derivative spectrophotometric method [6] using similar sample solutions as in the ^{19}F NMR experiments at 21 ± 2 °C (FNZ did not show distinct spectral change upon the addition of PC SUV).

The derivative intensity change (ΔD) caused by the addition of PC vesicles was measured at $\lambda =$ 265 nm for TFPZ and at $\lambda = 245$ nm for FZ, respectively, and from the relationship between ΔD value and the PC concentration, the K_p-values of TFPZ and FZ were calculated [6,12] and showed in Table 1. The K_p-values obtained by the second-derivative spectrophotometric method support the results of the ^{19}F NMR method.

The fraction of each drug in the PC bilayer was calculated from Eq. (7) with the obtained K_p- and $\Delta\delta_{max}$-values, and the results are shown in Fig. 3(A), (B) and (C) for TFPZ, FNZ and FZ, respectively. The experimental values of the fraction bound of these drugs are also plotted in Fig. 3 and show a good correlation with the calculated curves.

Conclusively, it can be emphasized that when the fluorinated drugs do not have absorption in the ultraviolet or visible regions, or having absorption but do not show significant changes according to their partition to lipid bilayers, ^{19}F NMR can be usefully applied to the determination of their K_p-values without any separation procedure that may disturb the equilibrium state of the sample solution. Moreover, the proposed method sidesteps the problem of the background signal interferences since the natural biological substances do not include fluorine atom(s).

As there are several kinds of widely administrated drugs containing fluorine atom(s), the proposed ^{19}F NMR method will be advantageously used to obtain their partition coefficients between phospholipid liposomes and water.

References

[1] Y.W. Choi, J.A. Rogers, Pharm. Res. 7 (1990) 508–512.
[2] J.A. Rogers, Y.W. Choi, Pharm. Res. 10 (1993) 913–917.
[3] B. Castro, P. Gamerio, J.L.F.C. Lima, C. Matos, S. Reis, Lipids 36 (2001) 89–96.
[4] C. Ottiger, H. Wunderli-Allenspach, Pharm. Res. 16 (1999) 643–650.
[5] C. Ottiger, H. Wunderli-Allenspach, Eur. J. Pharm. Sci. 5 (1997) 223–231.

[6] K. Kitamura, N. Imayoshi, T. Goto, H. Shiro, T. Mano, Y. Nakai, Anal. Chim. Acta 304 (1995) 101–106.
[7] S. Takegami, K. Kitamura, T. Kitade, K. Hasegawa, A. Nishihira, J. Colloid Interf. Sci. 220 (1999) 81–87.
[8] P.N. Venkatasubramanian, Y.J. Shen, A.M. Wyrwicz, Biochim. Biophys. Acta 1245 (1995) 262–268.
[9] P. Tang, B. Yan, Y. Xu, Biophys. J. 72 (1997) 1676–1682.
[10] K. Kitamura, M. Yamamoto, S. Takegami, M. Sugiura, Talanta 49 (1999) 261–265.
[11] A.J. Christopher, T.R.F.W. Fennell, Microchem. J. 12 (1967) 593–605.
[12] A.A. Omran, K. Kitamura, S. Takegami, A.Y. El-Sayed, M. Abdel-Mottaleb, J. Pharm. Biomed. Anal. 25 (2001) 319–324.