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ScienceDirect
International Journal of Pharmaceutics 352 (2008) 217–224
INTERNATIONAL JOURNAL OF PHARMACEUTICS
www.elsevier.com/locate/ijpharm

Mechanistic analysis of pH-dependent solubility and trans-membrane permeability of amphoteric compounds: Application to sildenafil

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Received 30 January 2007; received in revised form 26 October 2007; accepted 28 October 2007
Available online 4 November 2007

Abstract

This study was aimed at investigating the pH-dependent solubility and in vitro transmucosal permeability of sildenafil, an amphoteric compound with limited aqueous solubility, across parallel artificial membrane. The aqueous solubility and permeability of sildenafil as a function of solution pH were theoretically derived from the individual contributions of all species (cationic, neutral and anionic). The stability, octanol–water distribution coefficient ($\log D$), and solubility of sildenafil were then determined at various pHs, the permeability study was also performed at different pHs using parallel artificial membrane. The pH-solubility and -permeability profiles were then fitted to theoretical equations using non-linear regression.

The experimental pH-solubility profile was fitted very well to the theoretical equations ($R^2 = 0.9996$). The in vitro permeability of saturated sildenafil solution at different pH values also showed similar trend as the predicted one ($R^2 = 0.7829$). The two optimum pH (pH_{max}) values were found to be 4.50 and 10.24, where the maximum solubility of either cationic or neutral species, or anionic and neutral species is simultaneously obtained, and the maximal transmucosal fluxes (J_{ss}) are achieved. The above method can be applied to optimize the transmucosal delivery of other amphoteric drugs with low aqueous solubility.

Keywords: Sildenafil; Solubility; Sublingual; Permeability; pH; Parallel artificial membrane

1. Introduction

Sildenafil, a selective inhibitor of phosphodiesterase type 5 enzyme (PDE5), is extensively used for the treatment of erectile dysfunction (ED) (Boolell et al., 1996a,b). At present, sildenafil can only be administrated by oral route (Chan-Tack (1998); Goldstein et al., 1998). There are two disadvantages of oral sildenafil: (1) the bioavailability and pharmacological activity of oral sildenafil are notably affected by gastric empty and first-pass metabolism, the absolute bioavailability of 50 mg oral dose was 41% in healthy male subjects; drug absorption was delayed by food (Nichols et al., 2002). (2) Delayed pharmacological effect was also reported during sex intercourse, the onset of action usually started after 30–45 min after dosing (Eardley et al., 2002).

Sublingual mucosal delivery can offer attractive therapeutic advantages. The sublingual mucosa consists of thin non-keratinized, stratified epithelium that allows drug permeation to reach 4–4000 times greater than that of the skin (Shojaei et al., 1998). Moreover, the enzymatic degradation in the gastrointestinal tract and first-pass metabolism in the liver can be avoided (Squier and Hall (1985)). Thus, sublingual drug administration can potentially achieve high bioavailability and rapid onset of desirable action in a convenient manner.

Sublingual delivery of sildenafil has been clinically investigated (De Siati et al., 2003; Deveci et al., 2004). Results showed that the onset of erection with sublingual sildenafil could be effectively shortened to 15.5 min and not affected by food ingestion. Thus, transmucosal delivery (i.e. sublingual, buccal, and intranasal) of sidenafil may be potentially selected as alternative routes to bypass first-pass metabolism and achieve rapid onset of action as well as high bioavailability.

In these studies, however, only conventional oral tablets were utilized without formulation optimization suitable for sublingual administration. In fact, the absorption rate and pharmacological behavior of sildenafil are markedly influenced by its physicochemical properties (i.e. lipophilicity, molecular weight, pK_a, saliva stability and solubility, etc.) as well as formulation factors

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doi:10.1016/j.ijpharm.2007.10.039

such as drug release rate from formulation. As a high lipophilic compound, it can be quickly absorbed by sublingual mucosa. However, the low solubility in saliva seems to be a limiting factor for its absorption rate via sublingual mucosa (Rathbone et al., 2004). In our preliminary investigation, sildenafil solubility is below 1 mg in human saliva with pH 6.5–6.9, therefore after sublingual administration of sildenafil tablet, the drug is quickly saturated in saliva, which may result in the slow absorption rate. To improve the efficiency of sublingual absorption, the solubility of sildenafil in saliva should be maximized. Meanwhile, sildenafil aqueous solubility is greatly affected by the solution pH. As an amphoteric compound, both weakly acid centre and weakly basic centre exist in sildenafil molecule (Gobry et al., 2000), the acid–base equilibrium of sildenafil is depicted in Fig. 1. The neutral and ionized species show different lipophilicity, aqueous solubility, and permeability, therefore the fraction of these species may markedly influence sildenafil permeability across mucosa. Therefore, a mechanistic analysis of solubility and permeability at different pHs will facilitate to optimize transmucosal delivery of sildenafil and other ionizable drugs with low aqueous solubility.

2. Theory

2.1. pH-solubility profile

As an amphoteric electrolyte, two equilibriums for sildenafil (N) simultaneously coexist in aqueous solution at different pHs.

As weak acid

$$NH^+ + H_2O \overset{K_{a1}}{\rightleftharpoons} N + H_3O^+ \quad (1)$$

As weak base

$$N + H_2O \overset{K_{a2}}{\rightleftharpoons} N^- + H_3O^+ \quad (2)$$

The two dissociation constants are defined as

$$K_{a1} = \frac{[N][H_3O^+]}{[NH^+]} \quad (3)$$

$$K_{a2} = \frac{[N^-][H_3O^+]}{[N]} \quad (4)$$

The pH-solubility profiles of weakly basic and weakly acidic compounds in aqueous solution have been investigated by Kramer and Flynn (1972) and Ledwidge and Corrigan (1998). These theoretical equations can also be derived and applied in describing pH-solubility of amphoteric compounds. Briefly, for certain amphoteric compounds, the total solubility (S_T) at arbitrary pH is the sum of the concentration of the cationic species (NH^+), neutral species (N) and anionic species (N^-).

$$S_T = [NH^+] + [N] + [N^-] \quad (5)$$

At extreme low pH, the cationic species is saturated, and its intrinsic solubility is defined as S_+, the concentrations of neutral species and cationic species are determined by pK_a and S_+. According to Eqs. (3)–(5), S_T could be obtained as a function of S_+ and pH ($[H_3O^+]$):

$$S_T = S_+ + S_+\frac{K_{a1}}{[H_3O^+]} + S_+\frac{K_{a1}K_{a2}}{[H_3O^+]^2} = S_+\left(1 + \frac{K_{a1}}{[H_3O^+]} + \frac{K_{a1}K_{a2}}{[H_3O^+]^2}\right) \quad (6)$$

Similarly, at moderate pH between pK_{a1} and pK_{a2}, the neutral species is saturated and its intrinsic solubility is defined as S_N, S_T is therefore expressed as a function of S_N and pH:

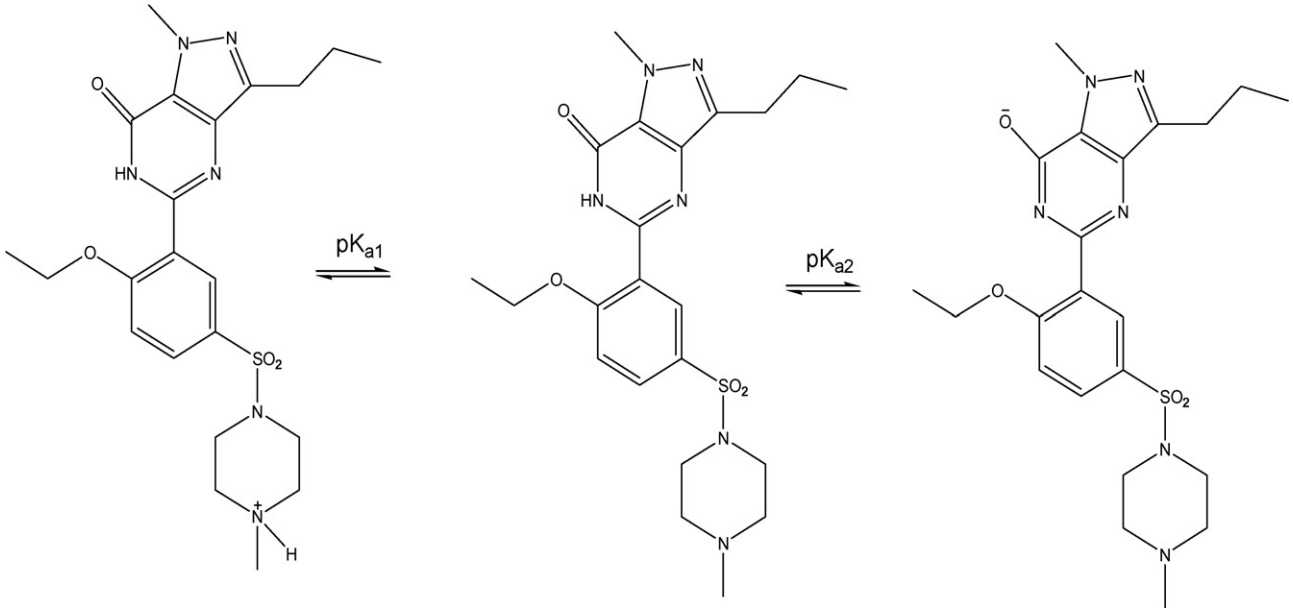

Fig. 1. Ionization equilibrium of sildenafil (Gobry et al., 2000).

$$S_T = S_N\frac{[H_3O^+]}{K_{a1}} + S_N + S_N\frac{K_{a2}}{[H_3O^+]} = S_N\left(1 + \frac{[H_3O^+]}{K_{a1}} + \frac{K_{a2}}{[H_3O^+]}\right) \quad (7)$$

At one particular pH (defined as pH_{max1}), the concentrations of both the cationic species and neutral species may simultaneously reach their intrinsic solubilities. When pH is lower than pH_{max1}, the cationic species is saturated, whereas the concentration of the neutral species is determined by the solution pH and S_- according to Eq. (2); when pH higher than pH_{max1}, the neutral species is saturated (S_N) and the concentration of cationic species is determined by pH and S. Therefore pH_{max1} can be calculated by rearranging Eq. (3):

$$pH_{max1} = -\log\left(\frac{K_{a1}S_+}{S_N}\right) = pK_{a1} - \log\frac{S_+}{S_N} \quad (8)$$

At extreme high pH, the anionic species is saturated (its intrinsic solubility is defined as S_-), then S_T can be determined by S_- and pH:

$$S_T = S_-\left(1 + \frac{[H_3O^+]}{K_{a2}} + \frac{[H_3O^+]^2}{K_{a1}K_{a2}}\right) \quad (9)$$

There should also exist a pH_{max2}, where both neutral and anionic species simultaneously reach their intrinsic solubilities.

$$pH_{max2} = pK_{a2} + \log\frac{S_-}{S_N} \quad (10)$$

2.2. Transmucosal permeability as a function of pH

Sildenafil molecules transported across the mucosal membrane include cationic species, neutral species and anionic species. The apparent steady-state flux (J_{ss}) is the sum of the steady-state fluxes of three species:

$$J_{ss} = J_+ + J_N + J_- \quad (11)$$

where J_+, J and J_- refer to the flux of cationic species, neutral species and anionic species, respectively. The individual steady-state flux (J_{ss}) of three species can be expressed by the following equations:

$$J_+ = \frac{dQ_+}{dt \cdot A} = P_+C_+ \quad (12)$$

$$J_N = \frac{dQ_N}{dt \cdot A} = P_NC_N \quad (13)$$

$$J_- = \frac{dQ_-}{dt \cdot A} = P_-C_- \quad (14)$$

where Q_+, Q_N and Q_- are the accumulated amounts of three individual species that penetrated across the membrane, P_+, P_N and P_- are the permeability coefficients of three species and C_+, C_N and C_+ are the individual concentrations of the three species in solution, respectively. A is the area of membrane. P_+, P_N and P_- are all independent of solution pH.

Therefore

$$J_{ss} = P_+C_+ + P_NC_N + P_-C_- \quad (15)$$

From Eq. (15), the apparent permeability coefficient (P_{app}) can also be viewed as the co-contribution of all three species:

$$P_{app} = \frac{J_{ss}}{C_0} = P_+X_+ + P_NX_N + P_-X_- \quad (16)$$

where C_0 is the initial sildenafil concentration and X_+, X_N and X_- are the fractions of the three species in solution, respectively. The J_{ss} of sildenafil at different pHs can be expressed as the function of solution pH by combining Eqs. (6), (7), (9) and (15):

When $pH \le pH_{max1}$

$$J_{ss} = S_+\left(P_+ + P_N\frac{K_{a1}}{[H_3O^+]} + P_-\frac{K_{a1}K_{a2}}{[H_3O^+]^2}\right) \quad (17)$$

When $pH_{max1} \le pH \le pH_{max2}$

$$J_{ss} = S_N\left(P_+\frac{[H_3O^+]}{K_{a1}} + P_N + P_-\frac{K_{a2}}{[H_3O^+]}\right) \quad (18)$$

When $pH \ge pH_{max2}$

$$J_{ss} = S_-\left(P_+\frac{[H_3O^+]^2}{K_{a1}K_{a2}} + P_N\frac{[H_3O^+]}{K_{a2}} + P_-\right) \quad (19)$$

3. Materials and methods

3.1. Materials

Sildenafil citrate was a gift from Pfizer Inc., Hong Kong. Potassium dihydrogenphosphate, sodium dihydrogenphosphate, phosphoric acid, sodium hydroxide, 1-octanol, hexane and hexadecane were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). Acetonitrile (HPLC grade) was obtained from Lab Asian Co. (Bangkok, Thailand). MultiScreen Permeability Plate Assembly and 96-well PTFE Acceptor Plate were ordered from Millipore Ltd., Hong Kong.

3.2. Methods

3.2.1. Stability under various pHs

Sildenafil citrate was dissolved in 50 ml of deionized water to a final concentration of 20 μg/ml, and then divided into three vials, the pHs of each portion were adjusted to 3, 7 and 11 by 85% phosphoric acid or 20% sodium hydroxide. The vials were then incubated in a water bath at 37 °C, 1 ml sample was taken at fixed intervals, sildenafil concentration was analyzed by a validated HPLC method (Nagaraju et al., 2003). Briefly, the HPLC system consists of a Waters 600 controller, a 717_{plus} auto-sampler, a 2487 dual λ absorbance detector and a Thermo Hypersil-Keystone Column (250 mm × 4.6 mm, 5 μ Hypersil BDS C_{18}). The mobile phase consists of 33% acetonitrile and 67% 50 mM KH_2PO_4 (pH 3.0) at a flow rate of 1 ml/min. Chromatograms were recorded at 230 nm.

Table 1
Sildenafil solubility at different pH levels ($n = 4$)

	pH								
	3	4	5	6	7	8	9	10	11
Solubility (mg/ml)	6.965	7.077	2.068	0.114	0.025	0.027	0.040	0.103	0.322
S.D.	0.092	0.047	0.042	0.001	0.001	0.001	0.001	0.001	0.058

The assay showed excellent linearity in calibration curve ($R^2 = 0.9996$) in the experimental concentration range. The limit of detection is 10 ng/ml. The inter-day and intra-day precisions are less than 3%.

3.2.2. pH-solubility profile measurement

The pH-solubility profile of sildenafil was determined according to the method described by Li et al. (2005). Briefly, excess amount of sildenafil citrate powder and 10 ml deionized water were added to a 15 ml vial, the pH values of the suspensions were stepwise titrated to 3–11. The vials were then immersed in a shaking water bath at 37 °C and equilibrated for 48 h. After confirming the pH, the suspensions were then filtered through 0.2 μm syringe filters (Iwaki Glass, Japan). An aliquot of the filtrate was appropriately diluted, and then sidenafil concentration analyzed by HPLC described above.

3.2.3. Distribution coefficients at different pHs

1-Octanol and deionized water were pre-equilibrated at room temperature (20 °C) for 24 h. After separation of the two phases, exactly 3 ml of octanol was transferred into a screw capped glass tube, and then mixed with the 3 ml sildenafil solution (20 μg/ml in 0.05 M phosphate buffer) with various pHs at room temperature. The two phases were allowed to continuously equilibrate using tube rotator. After 24 h, the phases were left to separate and the concentration of sildenafil in aqueous phase was measured by HPLC according the method described above. Each experiment was performed in four replicates. The distribution coefficient was determined according to Eq. (20):

$$\log D = \log \frac{(C_0 - C_w)V_w}{C_w V_{oct}} \tag{20}$$

where C_0 and C_w are sildenafil aqueous concentration before and after partition experiment, respectively. V_w and V_{oct} are the volume of aqueous phase and octanol phase, and $V_w = V_{oct} = 3$ ml, therefore $\log D$ can be obtained as follows:

$$\log D = \log \frac{C_0 - C_w}{C_w}$$

The $\log D$ values at various pHs were also calculated using Advanced Chemistry Development (ACD/Labs) Software V8.14 for Solaris (1994–2007 ACD/Labs).

3.2.4. Permeability studies

The permeability study was performed using a MultiScreen Filter Plate Assembly. Before the assay, the artificial membrane solution was prepared by mixing 5 ml of hexadecane with 95 ml of hexane. 30 μl of the mixture were then applied to the centre of each filter well of the donor plate and the plate was placed in fume hood for 2 h till the membrane layer formed. 150 μl of saturated sildenafil solution with various pHs was transferred into donor chamber and 250 μl PBS (pH 7.4) were added to receiving wells. After incubation at 37 °C for 5 h, the drug concentrations in receiving wells were analyzed by HPLC using the method described above. The trans-membrane steady-state flux (J_{ss}) and the apparent permeability coefficients were calculated according to Eqs. (11) and (16).

4. Results

4.1. Stability under various pHs

The stability of sildenafil solutions at pH 3, 7 and 11 were investigated, results indicted that the remaining fractions of sildenafil at above three pH levels were all higher than 98% after 12 h of incubation (37 °C).

4.2. pH-solubility profile

The solubility of sildenafil at different pH levels is listed in Table 1. At pH 3.0, $[H_3O^+] = 10^{-3}$ M, $S_T = 6.965$ mg/ml, and $pK_{a1} = 6.78$, $pK_{a2} = 9.12$[10], therefore, Eq. (6) becomes

$$S_T = S_+ \left(1 + \frac{K_{a1}}{[H_3O^+]} + \frac{K_{a1}K_{a2}}{[H_3O^+]^2}\right)$$

$$= S_+ \left(1 + \frac{10^{-6.78}}{10^{-3}} + \frac{10^{-6.78} \times 10^{-9.12}}{[10^{-3}]^2}\right) = 1.00016 S_+$$

Therefore

$$S_+ = 6.964 \text{ mg/ml}$$

Similarly, at pH 11, $[H_3O^+] = 10^{-11}$ M, $S_T = 0.322$ mg/ml, according to Eq. (9):

$$S_T = S_- \left(1 + \frac{[H_3O^+]}{K_{a2}} + \frac{[H_3O^+]^2}{K_{a1}K_{a2}}\right)$$

$$= S_- \left(1 + \frac{10^{-11}}{10^{-9.12}} + \frac{10^{-22}}{10^{-6.78} \times 10^{-9.12}}\right) = 1.0132 S_-$$

Therefore,

$$S_- = 0.318 \text{ mg/ml}$$

At pH 8, $[H_3O^+] = 10^{-8}$ M, $S_T = 0.027$ mg/ml, from Eq. (7):

$$S_T = S_N\left(1 + \frac{[H_3O^+]}{K_{a1}} + \frac{K_{a2}}{[H_3O^+]}\right)$$
$$= S_N\left(1 + \frac{10^{-8}}{10^{-6.78}} + \frac{10^{-9.12}}{10^{-8}}\right) = 1.1361 S_N$$

Then

$$S_N = 0.024\,\text{mg/ml}$$

According to Eqs. (8) and (10), pH_{max1} and pH_{max2} can be obtained as:

$$pH_{max1} = pK_{a1} - \log\frac{S_+}{S_N} = 6.78 - \frac{6.964}{0.024} = 4.50$$

$$pH_{max2} = pK_{a2} + \log\frac{S_-}{S_N} = 9.12 + \log\frac{0.318}{0.024} = 10.24$$

And Eqs. (6), (7) and (9) becomes

When $pH \leq 4.50$

$$S_T = 6.964\left(1 + \frac{10^{-6.78}}{[H_3O^+]} + \frac{10^{-15.80}}{[H_3O^+]^2}\right) \quad (21)$$

When $4.50 \leq pH \leq 10.24$

$$S_T = 0.024\left(1 + \frac{[H_3O^+]}{10^{-6.78}} + \frac{10^{-9.12}}{[H_3O^+]}\right) \quad (22)$$

When $pH \geq 10.24$

$$S_T = 0.318\left(1 + \frac{[H_3O^+]}{10^{-9.12}} + \frac{[H_3O^+]^2}{10^{-15.80}}\right) \quad (23)$$

Both experimental pH-solubility profile of sildenafil and theoretically predicted one in terms of Eqs. (6)–(8) are plotted in Fig. 2, and the experimental data were fitted to theoretical Eqs. (6)–(8) using non-linear regression (GraphPad Prism® 4.0, GraphPad Software Inc., USA), the r^2 value was 0.9996.

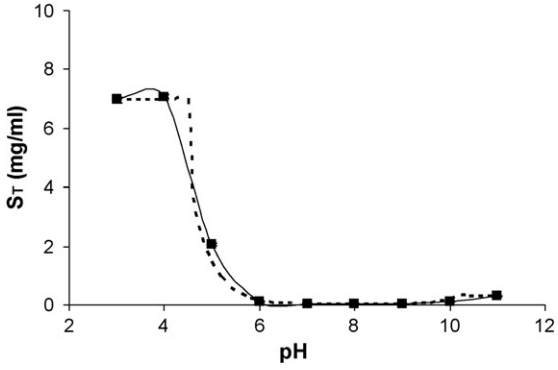

Fig. 2. pH-solubility profile of sildenafil (■) (mean ± S.D., $n = 4$) and comparison to the theoretical curve (dash line) using Eqs. (21)–(23).

4.3. Sildenafil log D at various pHs

The distribution coefficients (log D) of sildenafil at pH 3, 7 and 11 were measured. The individual log D of cationic, neutral and anionic species, which dominate at pH 3, 7 and 11, were estimated to be -0.52 ± 0.02, 1.59 ± 0.08, and 1.13 ± 0.02, respectively. The calculated log D values using ACD/Labs were -0.52, 2.24, and 2.01 at pH 3, 7 and 11, respectively. Both experimental and theoretical results indicted the lipophilicity of different species are in the order of neutral > anionic > cationic, and inversely proportional to their solubilities.

4.4. Permeability studies

The apparent permeability coefficient (P_{app}) of sildenafil at various pHs are shown in Fig. 3. Eqs. (3) and (4) can be future rearranged as:

$$\frac{[NH^+]}{[N]} = 10^{pK_{a1} - pH} \quad (24)$$

$$\frac{[N^-]}{[N]} = 10^{pH - pK_{a2}} \quad (25)$$

Therefore, at pH 3, the ratio of three species ($[NH^+]:[N]:[N^-] = 10^{3.78}:1:10^{-6.12}$), indicating that the concentration of both neutral and anionic species can be neglected. The permeability coefficient of cationic species (P_+) can be regarded as equal to the P_{app} at pH 3, or:

$$P_+ = 2.47 \times 10^{-8}\,\text{cm/s}$$

At pH 11, $[NH^+]:[N]:[N^-] = 10^{-4.22}:1:10^{1.88}$, according to Eqs. (24) and (25), so the permeability coefficient of anionic species (P_-) is roughly equal to P_{app} at pH 11:

$$P_- = 1.07 \times 10^{-5}\,\text{cm/s}$$

At pH 8, $P_{app} = 1.61 \times 10^{-5}$ cm/s, the fractions of three species are: $X_+ = 0.053$, $X = 0.880$, $X_- = 0.067$, and then the permeability coefficient of cationic species (P_N) can be calculated from Eq. (16):

$$P_N = 1.75 \times 10^{-5}\,\text{cm/s}$$

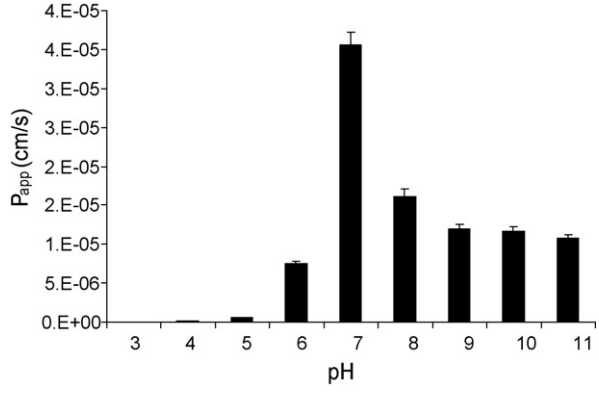

Fig. 3. P_{app} of sildenafil across parallel artificial membrane at various pHs (mean ± S.D., $n = 4$).

According to Eqs. (17)–(19), the pH-dependent J_{ss} of sildenafil can be expressed as follows:

When $pH \leq 4.50$

$$J_{ss} = 6.964\left(2.47\times10^{-8} + 1.75\times10^{-5}\frac{10^{-6.78}}{[H_3O^+]} + 1.07\times10^{-5}\frac{10^{-15.80}}{[H_3O^+]^2}\right) = 1.72\times10^{-7} + \frac{2.02\times10^{-11}}{[H_3O^+]} + \frac{1.18\times10^{-20}}{[H_3O^+]^2}\ (mg/cm^2/s) \quad (26)$$

When $4.50 \leq pH \leq 10.24$

$$J_{ss} = 0.024\left(2.47\times10^{-8}\frac{[H_3O^+]}{10^{-6.78}} + 1.75\times10^{-5} + 1.07\times10^{-5}\frac{10^{-9.12}}{[H_3O^+]}\right) = 3.57\times10^{-3}[H_3O^+] + 4.2\times10^{-7} + \frac{1.95\times10^{-16}}{[H_3O^+]}\ (mg/cm^2/s) \quad (27)$$

When $pH \geq 10.24$

$$J_{ss} = 0.318\left(2.47\times10^{-8}\frac{[H_3O^+]^2}{10^{-15.8}} + 1.75\times10^{-5}\frac{[H_3O^+]}{10^{-6.78}} + 1.07\times10^{-5}\right) = 4.96\times10^{7}[H_3O^+]^2 + 33.53[H_3O^+] + 3.40\times10^{-6}\ (mg/cm^2/s) \quad (28)$$

The experimental and theoretically predicted J_{ss} values of sildenafil at various pH levels are depicted in Fig. 4. These observed J_{ss} values were also fitted to the theoretical Eqs. (17)–(19) using non-linear regression and the r^2 value was 0.7829.

5. Discussion

In this study, the overall solubility and permeability of sildenafil across artificial membrane are considered as co-contributions from cationic, neutral and anionic species. Their intrinsic solubility and permeability coefficients are not altered by solution pH, the fractions of these species, therefore, were determined by pH and pK_a, the overall solubility and permeability can be derived as the function of pH.

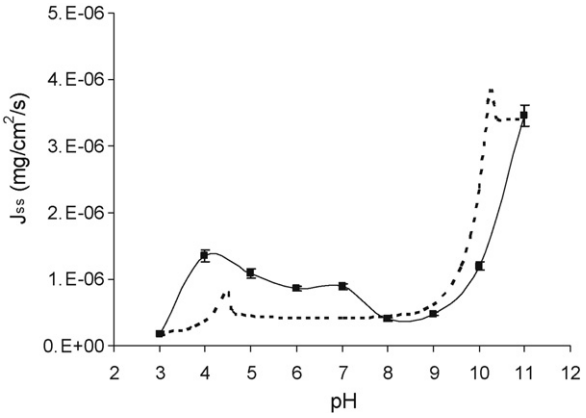

Fig. 4. Observed J_{ss} of sildenafil (■) across parallel artificial membrane at various pHs and in comparison to the theoretical curve (dash line).

To mechanistically analyze and quantitatively optimize the permeability of sildenafil, the constant parameters summarized in Table 2 are calculated from the experimental data, and then applied to theoretical equations to demonstrate both the solubility and steady-state flux across artificial membrane as a function of pH. Anionic species showed much higher lipophilicity as well as trans-membrane permeability than cationic species. Such phenomena can be explained by the different polarities between cation and anion. In Fig. 1, the negative change can be delocalized from on O-atom to the hyper-conjugation system in pyrimidine ring and other vicinal heterocyclic groups (p–π conjugation). The positive charge on cation, however, cannot be delocalized since no conjugation system exists nearby. Thus the anion exhibits smaller dipole moment and lower polarity than cation. The various lipophilicities of cationic, neutral and anionic species can well explain the different trans-membrane permeabilities at different pHs.

Gobry et al. (2000) investigated the conformational property of sildenafil using molecular-dynamics simulations. Their results indicated the capacity of cationic sildenafil to form intra-molecular H-bonds between one O-atom of the sulfonamide function and the proton of the basic N-atom. For the anionic molecule, however, H-bond cannot form internally. Therefore, they believed the lipophilicity of cation is much higher than that of the anion. Unfortunately, the experimental log D data on all three species were not available, and the influence of H-bond on molecular polarity is also questionable. Such inconsistency should be further investigated in future studies.

Table 2
Individual physiochemical properties of cationic, neutral and anionic species of sildenafil

	Species		
	Cationic	Neutral	Anionic
Solubility (mg/ml)	6.964	0.024	0.318
Lipophilicity (log D)	−0.52	∼1.59	1.13
Permeability coefficient (cm/s)	2.47×10^{-8}	1.75×10^{-5}	1.07×10^{-5}

In general, the lipophilicity of ionizable species is mainly determined by their chemical structure. However, the effect of ion pair formation may also be taken into account since ionized species of a compound form ion pair with counter ions in aqueous and then distribute to the octanol phase as ion pair (Roda et al., 1990). Therefore, the ionic strength in aqueous solution can also affect the log D values of cationic and anionic species (Austin et al., 1998). In our study, sodium and phosphate ions in the buffer system may form ion pairs with cationic and anionic molecules, such effect should be further investigated in future studies. In present study, the primary purpose of measuring lipophilicity of different species is to relatively compare the results with other literature and then explain their different permeabilities across artificial membrane.

The mechanistic analysis of different molecule species of ionizable compounds has been carried out to investigate the oral mucosal permeation properties (Chen et al., 1999; Chetty et al., 2001). In these studies, however, only unsaturated drug solution was used and the concentration of neutral species and charged species can be easily obtained from the initial loading concentration and pH. To achieve the highest transmucosal flux, the pH should be adjusted to maximize the fraction of neutral species, which shows much higher lipophilicity and permeability than the charged species. In sublingual delivery for many low-soluble compounds, however, concentrated solution (i.e. suspension, spray) or fast dissolved tablets are frequently used to achieve high salival drug concentration and fast onset of action (Allen et al., 2005). Therefore, the aqueous solubility at different pHs should also be considered. In Fig. 4, the maximal flux did not appear when solution pH ranging between 7 and 8, when the fraction of neutral species was maximized. Due to the low solubility of neutral species as compared to the ionized species, an optimization between the permeability and solubility should be studied experimentally and theoretically. At pH_{max1} and pH_{max2} generated from solubility and pK_a, both neutral species and charged species are saturated at the same time, naturally, the J_{ss} is also maximized at these two pHs. This assumption were confirmed by the two peaks appeared around pH 4 and 11 in the experimental pH–J_{ss} curve (Fig. 4).

The parallel artificial membrane is currently utilized as a high throughput screening approach for assay of passive transport (Kansy et al., 1998; Sugano et al., 2004; Zhu et al., 2002). In this study, the artificial membrane is constructed of hexane/hexadecane, which is particularly beneficial for screening permeability at multiple pHs because the membrane is barely affected by pH (Wohnsland and Faller (2001)). In the permeability study, the experimental J_{ss} correlated well to the theoretical equations by non-linear regression (Fig. 4), which showed that the in vitro trans-membrane permeability of saturated sildenafil at various pHs can be predicted from its physicochemical parameters (solubility and pK_a) and the maximal fluxes at pH_{max1} at pH_{max2} and can also be easily predicted according to Eqs. (8) and (10). Such method can be further applied to more ionizable drugs with low aqueous solubility through transmucosal delivery.

Acknowledgement

This work was supported ITF Gant (ITS/174/00) from Innovation & Technology Commission, Hong Kong SAR.

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