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Preformulation Study of Saquinavir: pH-Dependent Solubility, Ionization and Partition Coefficients

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ABSTRACT

Solubility, ionization and lipophilicity of a drug molecule are known to affect its transportation, distribution and permeation properties. The determination of physicochemical properties of saquinavir (SQV) was carried out to design alternative or improve on the available dosage form(s) of the compound capable of circumventing the obvious challenges with current available formulation while improving bioavailability and compliance. Solubility, pKa and octanol-water partition coefficient were determined by UV spectrophotometry in different buffer systems (pH 3-11, ionic strength 0.10 M at 25 ºC) and in selected solvent systems: polysorbate 80, PEG 400 and glycerol. Log P was determined by shake flask method, solubility was measured by adding excess SQV in 10 ml of buffer or solvent system at 25 ºC for 48 h, centrifuged, supernatant filtered, diluted and absorbance recorded at 265 nm to obtain solubility from a pre-calibrated and validated Beer's plot. The pKa was estimated from the point of inflection of first derivative plot of ΔAbs/ΔpH versus pH, Handerson-Hasselbach, intrinsic solubility and Bates-Schwarzenbach methods. A maximum aqueous solubility (1358 µg/ml) was observed around the pKa value. PEG 400 (10 %v/v) improved the solubility of SQV (2756 µg/ml). The log P measurements show a value >1.0 in buffer systems (pH 3-11 at 25 ºC). The order of solubility of SQV for the solvents are PEG 400>polysorbate 80>glycerol. The pKa of SQV ranges from 6.998 to 7.15 in buffer system at 25 ºC, 0.1 M. The physicochemical parameters found in this study could serve as veritable tool for possible further development of topical SQV capable of improving bioavailability and compliance.

Keywords: *Preformulation study, saquinavir, pH, spectrophotometry, partition coefficient*

1. INTRODUCTION

The poor aqueous solubility of SQV can limit its gastrointestinal absorption which, together with other physicochemical properties, reduces oral bioavailability. This limitation can lead to failure during product development and formulation, and masking other properties that affect both pharmacokinetics and pharmacodynamics of SQV. Preformulation studies are aimed at designing drugs for optimum and effective delivery to improve bioavailability profile. In order to design drugs for effective delivery, physicochemical properties (aqueous solubility, dissolution rate, ionization potential, melting point, partition coefficient, compatibility with other excipients), anticipated drug dose, available dose and stability of formulation have to be ascertained. It can assist formulation scientist to identify optimum molecule, provide suitable vehicles for development and aid proper selection of formulation methods. Saquinavir, chemically (2S)-N-[(2S,3R)-4-[(3S)-3-(tert-butylcarbamoyl)-decahydroisoquinolin-2-yl]-3-hydroxyl-1-phenylbutan-2-yl]-2-(quinolin-2 ylformamido) butanediamide, is a peptidomimetic hydroxyethylamine highly specific HIV-1 and HIV-2 protease enzyme inhibitor used in the management of human immunodeficiency virus, HIV [1]. It is used alone or in combination with other antiretroviral drugs. Combination of SQV with two nucleoside analogues has been regarded as standard therapy in HIV infected patients [2, 3]. Such combination arose due to limited bioavailability [4] of 4-12 per cent, extensive presystemic metabolism by CYP3A4 in liver and intestine [4, 5], p-glycoprotein mediated SQV efflux system from absorption site [6] and low plasma concentration necessary to enhance $CD4^+$ T-cell counts [7]. The marketing of the drug as hard and soft gel capsules since late 1990s could not solve completely the problems of low bioavailability of SQV either. Although the combination of SQV with low dose ritonavir has successfully reduced the high frequency of administration of SQV every 8 hours to once- or twice-daily administration and increases the SQV steady state area under the curve (AUC) by more than 20-30 fold [8], current available formulation as mesylate salt has nonlinear pharmacokinetics and still depends on presence of high calorie, high fat meal [9] to increase gastrointestinal absorption [8]. To improve delivery and bioavailability profile of SQV, several investigations ranging from simple microemulsion [10] to self-emulsifying [11], self-micro-emulsifying [12] and self nano-emulsifying [9] drug delivery systems have been reported. The studies, however, did not take into consideration the pH-dependent solubility, partitioning and ionization potentials of SQV in various vehicles. Such limitations formed the backbone of the present studies which included the determination of pKa (which is physicochemical parameter that describe the degree of ionization of functional groups with respect to pH), solubility (which provides guide for dosage formulation especially for drugs with solubility limited absorption) and partition coefficient (which provide the idea of target

route and form of drug delivery and predicts accurately the absorption rate of compounds).

2. MATERIALS AND METHODS

2.1 Chemicals and Reagents

Saquinavir is a gift from ROCHE Pharmaceuticals, India. Polysorbate 80 (Kermel), polyethylene glycol 400 (Sigma Aldrich), glycerol (May & Baker) and other chemicals were of analytical grade and were used without further purification. The UV spectrophotometer (Jeenway 6405, England) was used for spectrophotometric analysis.

2.2 Methods

2.2.1 Preparation of Solutions Used for Different Studies

The various buffer solutions were prepared accordingly and pH adjusted by 0.1 M sodium hydroxide (pH 5-8 and 10-11) or 0.1 M hydrochloric acid (pH 3-4 and 9) using accurately weighed quantities of potassium hydrogen phthalate, potassium dihydrogen phosphate, sodium tetraborate or sodium bicarbonate [13,14] as the case may be. Different strengths of polysorbate 80, glycerol and PEG 400 were prepared by homogeneous dissolution or mixing with double distilled water. The ionic strengths of the buffer solutions were adjusted to 0.10 M with NaCl.

2.2.2 Partition Coefficient Measurement

The traditional shake flask method [15] was adopted in this determination. Buffer solutions of different selected pH 3-11 were used as the aqueous phase while n-octanol was used as non-aqueous phase for each determination. For each measurement, 1 mL of 1000 µg/mL solution of SQV in methanol was added to 10 mL of n-octanol mutually saturated with 9 mL of selected pH buffer solution in a separating funnel. The mixture was mixed vigorously and stirred on thermostatted shaker for 24 hours, centrifuged 4000 rpm for 30 mins and allowed to equilibrate for 2 hours [16]. The absorbance of the upper aqueous layer was taken at 265 nm using appropriate blanks. All determinations were carried out in triplicate. The apparent partition coefficient (P_{app}) was calculated from equation 1 [15], the standard free energy change ($\Delta\mu^o$) in J per mole was estimated from equation 2 [17] while the fraction of ionized SQV (α) at different pH was calculated from equation 3 [18].

$$P_{app} = [C_o - C_w]V_o/C_wV_w \quad - \quad - \quad - \quad - 1$$

where C_o is the total concentration of SQV, C_w is the concentration of SQV in aqueous phase, V_o and V_w are the volumes of non-aqueous and aqueous phases respectively.

$$\Delta\mu^o = -RT \text{ In } P \quad - \quad - \quad - \quad - \quad - 2$$

where P is the partition coefficient estimated from the intercept of the plot of $1/P_{app}$ against $[H^+]$ of buffer solutions.

$$P = P_{app}/(1-\alpha) \quad - \quad - \quad - \quad - \quad - 3$$

where α is the fraction of SQV ionized at different buffer conditions.

2.2.3 Determination of Dissociation Constant (pKa) of SQV

The UV spectrophotometric method was used for this determination [15]. An aliquot volume of a primary stock solution of SQV (100 µg/mL) prepared in methanol was transferred to different calibrated volumetric flask and diluted to 10 µg/mL with each of buffer solution (pH 3-11). The resulting solution was scanned in the UV range of 200-400 nm. The spectra were recorded and overlapped to determine wavelength of maximum change in absorbance at different pH. A first derivative absorbance curve was plotted and the point of inflection was obtained as the pKa. The value obtained by the method was compared with those obtained with Bates-Schwarzenbach [19] and thermodynamic solubility [20] methods, where Ka (and pKa) was estimated from equations 4 and 5 respectively.

$$pKa = p((\alpha_H\gamma_{Cl}) - \log[(D_{HA}-D)/(D-D_{A^-})] \quad - \quad - \quad -4$$

where pKa is acidity constant, $p((\alpha_H\gamma_{Cl})$ is an acidity function, D_{HA}, D_{A^-} and D are absorbance value of SQV in acid, base and buffer respectively.

$$pKa = pH \pm \log[S_o/(S_o-S)] \text{ for weak basic drugs} \quad - \quad -5$$

where S is the solubility of SQV at experimental pH, S_o is the solubility of unionized SQV in double distilled water and pKa estimated from the intercept of plot of $\log[S_o/(S_o-S)]$ against pH of buffer

2.2.4 Determination of Thermodynamic Solubility of SQV

The modified shake flask method [21] was used for the study. Excess SQV was shaken with 10 mL of each of double distilled water and different strengths of glycerol, PEG 400 and polysorbate 80 at 37 ºC on a mechanical orbital shaker for 48 h and centrifuged for 15 mins. The supernatants were filtered through Whatmann filter (#41), suitably diluted and assayed spectrophotometrically for SQV against appropriate blanks. The solubility was calculated from the pre-calibrated standard curve.

2.2.5 Spectrophotometric Assay of Saquinavir

The concentrations of SQV were quantitatively determined by UV/VIS spectrophotometer (Jeenway 6405, England). The samples were analyzed at maximum

wavelength of 265 nm. The calibration curve (absorbance versus drug concentration) was constructed by measuring standard solutions of the drug in methanol for every series of samples. Validation of the method was performed to ensure that the calibration curve between 2 and 20 µg/ml was in the linearity range of the assay and the coefficients of variation were less than 2.0 % both intra-day and inter-day.

3. RESULTS AND DISCUSSION

The results of the log P measurement are shown in Table 1 and Figure 3. The P_{app} and α values of SQV are pH-dependent as the lowest values of P_{app} and highest ionized fractions were recorded in acidic pH buffer systems.

Table 1: Results of Octanol/Buffer System Partitioning and Degree of Ionization of SQV

Buffer pH	P_{app}	α	% ionization
3	0.010	0.995	99.5
4	0.115	0.944	94.4
5	0.185	0.910	91.0
6	0.278	0.864	86.4
7	1.027	0.499	49.9
8	2.000	0.024	2.4
9	2.500	-	-
10	2.500	-	-
11	2.500	-	-

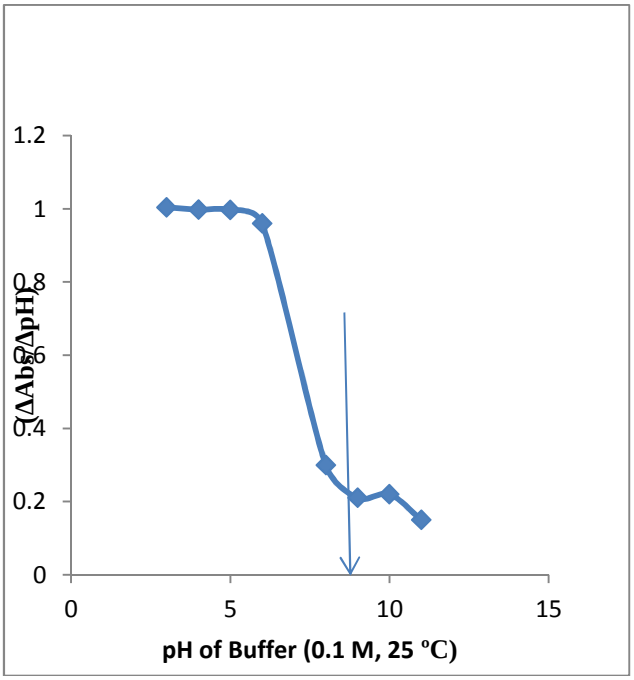

Fig 1: Spectrophotometric First Derivative Determination of pKa of SQV

The log P value of 2.05 was estimated from the intercept on plot of $1/P_{app}$ against the $[H^+]$ of buffer systems (Figure 3). This shows that SQV partition more in the non-aqueous phase and exist in ionized form in lower pH regions. The existence of SQV in the ionized form at lower pH could be attributed to the basic nature of drug (Figure 2) which ensures protonation of the amine functional group at pH below the pKa value of the drug by the highly electrophilic buffer systems.

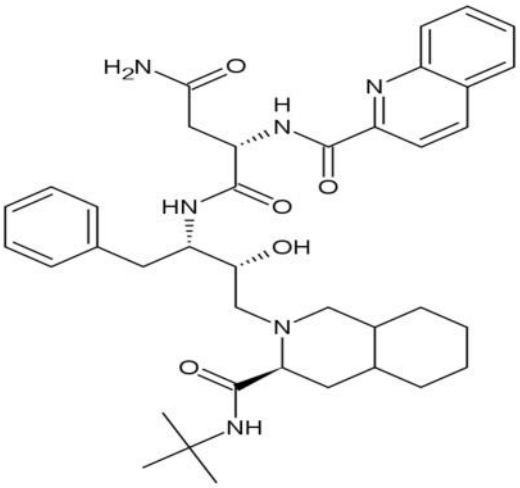

Fig 2: Chemical Structure of SQV

As the pH of the buffer approaches the pKa, the protonation decreases and then diminishes gradually at pH conditions above pKa of SQV. At the pKa region, the drug exists as zwitterions as shown by the nearly 50 % ionization at pH 7. The variation in log P follows similar trend as observed by the P_{app} value of 1 near the pKa region of SQV.

The determination of pKa of SQV is significant due to its effects on lipophilicity, solubility, absorption (via pH-partition theory), elimination incase of toxic overdoses (via pH control), interpretation of analytical data from HPLC and other techniques, drug-receptor bindings, and binding with membranes, membrane analogues and proteins. The pKa determined from various methods show similar values. The point of inflection from the spectrophotometric first derivative curve (Figure 1) gave pKa value of 7.15, the estimate from equation of line (Figure 3), where slope = 1/Ka.P gave value of 7.11.

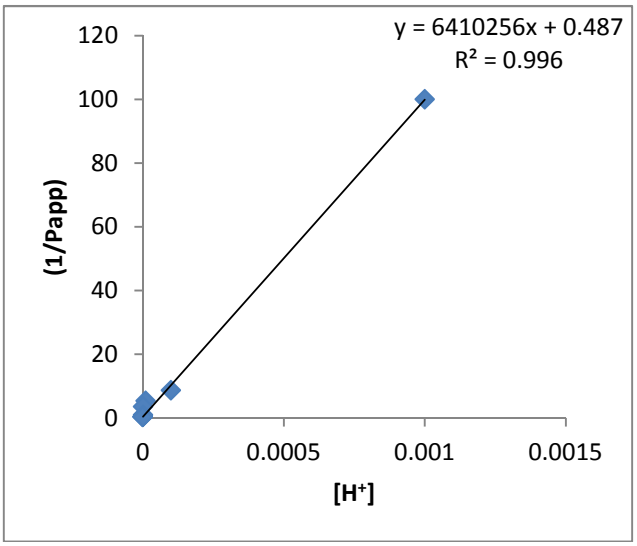

Fig 3: Partition Coefficient Measurement from Handerson-Hasselbach Equation

The estimates from Bates-Schwarzenbach (equation 4) and thermodynamic (intrinsic) solubility (equation 5) methods gave the pKa value of 7.09 and 6.998 respectively with the standard free energy change of -1778.50 Joule per mole showing the exothermic nature of dissolution and partitioning of SQV in solvents and vehicles.

Solubility has important effects on drug development and delivery. For ionizable chemical entity such as SQV, solubility is carried out at different pH to mimic the pH gradient observed in the gastrointestinal tract for intended orally administered drugs. SQV showed pH dependent solubility with improved aqueous solubility at low pH regions (Figure 4).

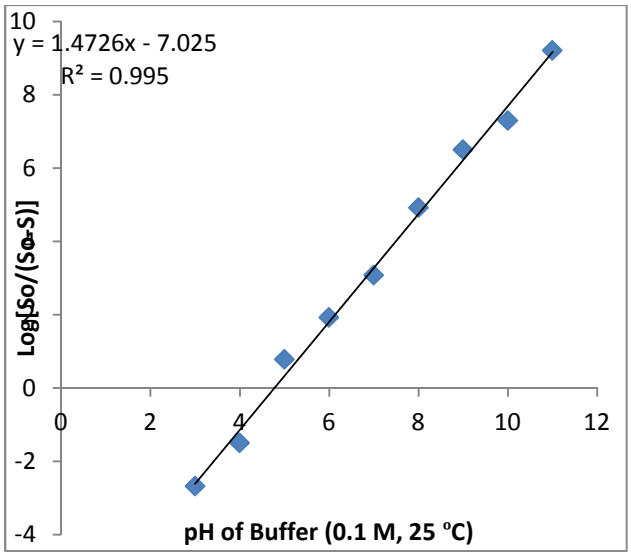

Fig 4: pH-Dependent Solubility of SQV in Buffer Solutions (0.1 M, 25 °C)

The effects of glycerol, polysorbate 80 and PEG 400 were carried out at pH where no ionization occurs to eliminate the influence of pH on solubility. It was observed that all the vehicles improved aqueous solubility of SQV with highest improvement (2756 µg/mL) in 10 %v/v PEG 400 compared with 1358 µg/mL, 2005 µg/mL and 1836 µg/mL in double distilled water, 10 %v/v polysorbate 80 and glycerol respectively (Figure 5).

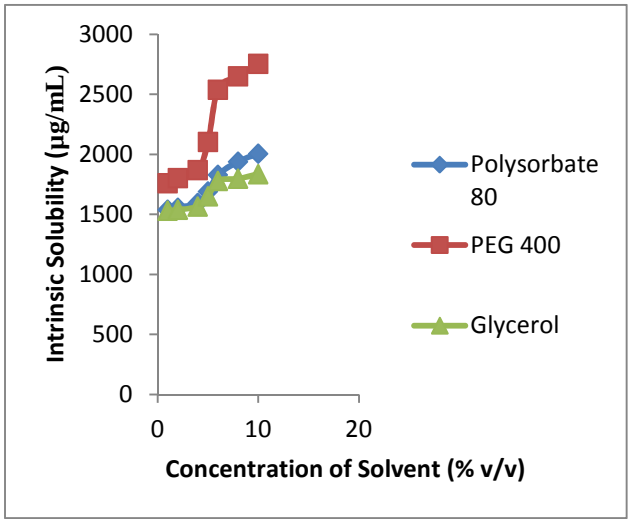

Fig 5: Effects of Selected Strengths of Vehicle on Solubility of SQV

Polysorbate 80 (a PEG-elated sorbitan esterified with fatty acid) improves solubility of poorly water soluble drugs by formation of micelles in which hydrophobic part will aggregate and its core surrounded by the polar head groups in aqueous phase due to its amphiphilic nature [22]. Another postulation attributed its solubilizing effect to the dispersant property of surfactants [23]. PEG 400, a polymer of ethylene oxide, enhances solubility via coupling to hydrophobic molecules to produce non-ionic surfactants as a result of hydrogen bonding. The variation in their extent of solubilizing effects on SQV could, however, be attributed to their dielectric constants differences.

4. CONCLUSION

The partition coefficient value determined is significant because it qualifies SQV for topical delivery (log P 1-3) for further development. The enhanced solubility of SQV in the vehicles studied showed that incorporation of polysorbate 80, PEG 400 or glycerol in formulation for oral and topical/transdermal delivery can improve bioavailability of SQV because polysorbate 80 has low toxicity and irritancy and can be used for oral and parenteral preparations, while glycerol is clear syrupy liquid with a sweet taste. Many biological processes can be correlated with the physicochemical properties of drugs such as absorption potential, membrane

permeability, plasma protein binding, volume of distribution, renal and hepatic clearance, binding to target enzymes and receptors, binding to plasma proteins, gastrointestinal absorption, central nervous system penetration and dissolution rate. The physicochemical properties determined from the studies provide promising insights into further development processes of SQV for effective delivery to target tissues and organs.

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