DRUG DEVELOPMENT AND INDUSTRIAL PHARMACY®
Vol. 30, No. 9, pp. 967–973, 2004

Solution Stability of Factor Xa Inhibitors as a Function of pH

Pei Yang* and Fraser Macdonald

Drug Development, Millennium Pharmaceuticals, Inc., Cambridge, Massachusetts, USA

ABSTRACT

The stability of two factor Xa inhibitors (MLN1021 and CT54004) as a function of pH was investigated in order to better characterize the compounds and to locate the optimum pH for a stable formulation that might be used in a first-in-person (FIP) clinical study. The compounds prepared in buffer solutions over a pH range of 1–10 were stored at 60°C, and assayed for pH and purity at different time points. The pH-rate profiles indicated that both compound was quite stable in the middle pH range, and least stable at pH<3 and >8. The optimum pH for stability of the compounds was around 6, which is consistent with the theoretical predictions by a mathematical model. The mechanism of the reaction is not a specific-acid/base-catalyzed reaction. Based on the experience of this study, an ideal experimental design is proposed, which will be useful for future study on similar drug candidate.

Key Words: Factor Xa; pH; Stability; Degradation rate.

INTRODUCTION

The drug stability has been investigated extensively.[1] The rate constant was used as a parameter to describe the time course of reactions. In aqueous solutions, the solution pH is often a key role in determining the rates of reactions, usually as a consequence of catalytic processes. The exploration on the pH dependence of reaction rates will shed light on the mechanism of the catalysis and provide very practical information about drug stability.[2]

Human factor Xa (fXa), a trypsin-like serine protease, is an attractive target for the development of new orally active anticoagulants as heparin and warfarin replacements. As novel therapies for thromboembolic disorders, fXa inhibitors play a central role in the coagulation cascade. Discovery and development of small molecule competitive factor Xa inhibitors has drawn significant efforts in the pharmaceutical industry.[3]

Our previous stress stability study on several factor Xa inhibitors indicate that the solution stability of the

*Correspondence: Pei Yang, Drug Development, Millennium Pharmaceuticals, Inc., 40 Landsdowne St., Cambridge, MA 02139, USA; Fax: (617) 551-3745; E-mail: peiyangusa@yahoo.com.

DOI: 10.1081/DDC-200037240
0363-9045 (Print); 1520-5762 (Online)

compounds was pH dependent. The study was conducted under widely different pH conditions (in 0.01 N NaOH containing 25% acetonitrile, 0.01 N HCl, or water). It was found that these compounds were most susceptible to degradation under basic conditions, less so in 0.01 N HCl, and most stable in water (where the solution pH is 6.0). It was of interest to examine more closely the stability of two lead compounds (MLN1021 and CT54004) as a function of pH in order to better characterize the compounds and to locate the optimum pH for a stable formulation that might be used in a first in person (FIP) clinical study.

In this study, MLN1021 and CT54004 were studied as the function of pH. The rate constants at different pH were obtained. The optimum pH for stability was determined by experiments and confirmed by a mathematical approach.

THEORETICAL SECTION

A kinetic model is established for pH-rate profiles. The expression uses the experimental rate constants to set the equation.[2]

$$\text{Assuming rate} = k[S]^p = k_1[S]^p[H^+]^n + k_2[S]^p + k_3[S]^p[OH^-]^m \quad (1)$$

where k, k_1, k_2 and k_3 are, respectively, the rate constants for the overall, the specific-acid-catalyzed, uncatalyzed, and specific-base-catalyzed reactions, S is a non-ionizable reactant, and p, m and n are the orders with respect to non-ionizable reactant, hydrogen ion and hydroxide ion, respectively. A general equation can be obtained as

$$\begin{aligned} k &= k_1[H^+]^n + k_2 + k_3[OH^-]^m \\ &= k_1[H^+]^n + k_2 + k_3(k_w/[H^+])^m \\ &= k_1[H^+]^n + k_2 + k_4/[H^+]^m \end{aligned} \quad (2)$$

where k_w is the ion product of water ($pK_w = 13.02$ at 60°C),[2] and k_4 is the rate constant of the specific-base-catalyzed reaction in terms of $[H^+]$ rather than $[OH^-]$.

At low pH and high pH, one term is much higher than other terms considering $[H^+]$ or $[OH^-]$ is large, and the general equation can be simplified into two equations for low pH and high pH, respectively.

$$\log k = \log k_1 - n\ pH \text{ for low pH} \quad (3)$$

$$\log k = \log k_4 + m\ pH \text{ for high pH} \quad (4)$$

These two equations are mathematical expressions for two straight-line segments at low and high pH. The intersection of the two lines on the log k-pH profile should be the condition where the drug is most stable.

Mathematically, the drug is most stable when the derivative of degradation rate (k′) equal to zero. The pH at the minimum of the log k-pH profile can be obtained by taking the derivative of Eq. 2, setting that derivative equal to zero,

$$k' = nk_1[H^+]^{n-1} - mk_4/[H^+]^{m+1} = 0 \quad (5)$$

and solving for pH_{min}:

$$\begin{aligned} pH_{min} &= \frac{1}{m+n}\log\frac{nk_1}{mk_4} \\ &= \frac{1}{m+n}\left[\log\frac{n}{m} + \log k_1 - \log k_4\right] \end{aligned} \quad (6)$$

Since the intercepts (log k_1 and log k_4) and the slopes ($-n$ and m) of the two straight lines can be obtained from the regression equations on log k-pH plot, this equation can be used to calculate the pH of maximum stability.

Ideally the slopes should be 1 and -1, which indicates that the reaction is specific-acid-base-catalyzed reaction, and the pH-degradation rate profile should be a V-graph.

METHODS

Preparation of Buffer Solutions

Buffer solutions ranging from pH 1 to 10 were prepared. Potassium Chloride (Aldrich, Milwaukee, WI) and Hydrochloride (Pierce, Rockford, IL) were

Table 1. Buffer concentrations of some selected buffer solutions at different pH.

pH	Buffer conc. (M)
1.0	0.16
3.0	0.12
4.0	0.14
4.5	0.15
5.0	0.15
5.5	0.16
6.0	0.16
6.5	0.17
7.0	0.18
8.0	0.20
10.0	0.10

used to prepare the buffer of pH 1.[4] Sodium phosphate dibasic heptahydrate and citric acid (Mallinckrodt, Paris, KY) were used to prepare the buffer of pH 3 to 8.[5] Sodium carbonate and sodium bicarbonate (J. T. Baker, Phillipsburg, NJ) were used to prepare the buffer of pH 10.[4] The buffer concentration at different pH ranged from 0.1 M to 0.2 M (see Table 1), providing enough buffer capacity in drug solution.

Preparation of API Stock Solution (~10 mg/mL)

Each of MLN1021 API and CT54004 API (synthesized by the Medicinal Chemistry group at Millennium South San Francisco Site) was used to prepare API stock solution. About 100 mg API was weighed out, dissolved in water, sonicated for 30 min, vortexed for 20–30 seconds, and qs to 10 mL in volumetric flask.

Preparation of Drug Solutions in Buffer (~1 mg/mL)

Drug solutions in buffer were prepared at about 1 mg/mL by diluting API stock solution ten-fold with each buffer solutions. Blank samples are treated in the same manner and diluted using the same dilution factors by pipetting DI water into the buffer solutions.

Sample Incubation

Samples were incubated at 60°C in incubator (model Imperial III, Lab-line Instruments, Melrose Park, IL), and the time was recorded at which the incubation period began.

Preparation of Quenched Drug Solutions (~0.1 mg/mL)

At predetermined time points, 100 μL of incubated solution was collected and immediately neutralized by adding 900 μL of pH 7 buffer solution (ten-fold dilution) in order to quench the degradation process. The solution was then vortexed for about 20–30 seconds prior to analyzing. The time points for analysis were between 0 and 24 hour for the section of the study conducted at lower pH and higher pH, while they were between 0 and 4–6 days for the section of the study conducted at pH 4 to 7.

pH Determination

The pH were determined for all buffer solutions, incubated drug solutions in buffer, and blank solutions at the initial time-point and final time-point, and for all quenched drug solutions using a calibrated pH meter (Model Φ72, Beckman Instrument, Fullerton, CA).

HPLC Analysis

The sample purity was analyzed using an HPLC system equipped with a UV detector (Model 996 Photodiode Array, Waters, Milford, MA) at 235 nm. The blank sample at different pH was also tested by HPLC, and its chromatogram was used to compare with those of drug sample by overlapping and subtracting. The HPLC system comprised a 150×4.6 mm, 5 μm C18 column (BetaBasic, Thermo Electron, Bellefonte, PA), a pump (Model 625, Waters), an autosampler (Model 717, Waters), and a computer with a Millennium^{32} data analysis software (Waters). The mobile phase consisted of 0.1% TFA in DI water (mobile phase A) and 0.85% TFA in acetonitrile (mobile phase B). The flow rate was 1.0 mL/min. The total run for each sample was 65 minutes by a gradient method. Since the sample was quenched, it should not be so critical on whether the sample is analyzed right away. However, in this study the samples were injected as close as possible to the time of sampling.

RESULTS AND DISCUSSION

pH Change

There was no significant pH change between solutions at the initial time-point and at the final time-point for buffer solutions, drug solutions in buffer,

Table 2. % Drug remaining at the last time-point at typical different pH.

Compound	Timepoint (hr)	pH of Drug solution	% Drug remaining
MLN1021	140	5.2	91.76
		5.8	96.09
		6.1	**96.66**
		6.6	96.48
CT54004	137	4.7	85.73
		5.1	91.23
		5.7	94.84
		5.8	95.32
		6.0	**95.59**
		6.3	95.01
		6.5	93.82

The bold font indicates the most stable condition.

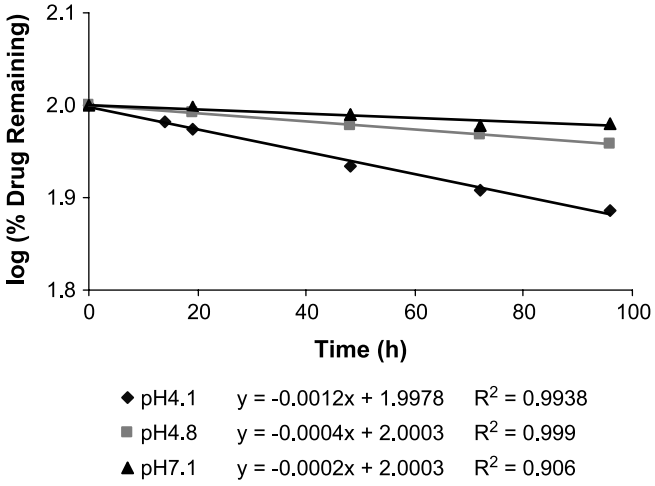

Figure 1. Typical degradation profiles (MLN1021 at pH 4.1, 4.8, and 7.1).

and blank solutions (data not shown). The pH values of the quenched drug solutions were within 6.8–7.1, indicating a successful neutralization.

Stability

The % drug remaining was calculated based on the sample purity (i.e., % peak area from the HPLC analysis). Table 2 summarized some typical data on % drug remaining at the last time-point at different pH for both compounds. The results show that, among all the investigated pH, the % drug remaining at the last time-point was highest at pH 6.1 for MLN1021 (96.66%) and at pH 6.0 for CT54004 (95.59%).

To estimate the degradation rate, the logarithm of % drug remaining was plotted versus time, and the data were fitted with linear regression. Figures 1 and 2 are typical degradation profiles at some pHs for MLN1021

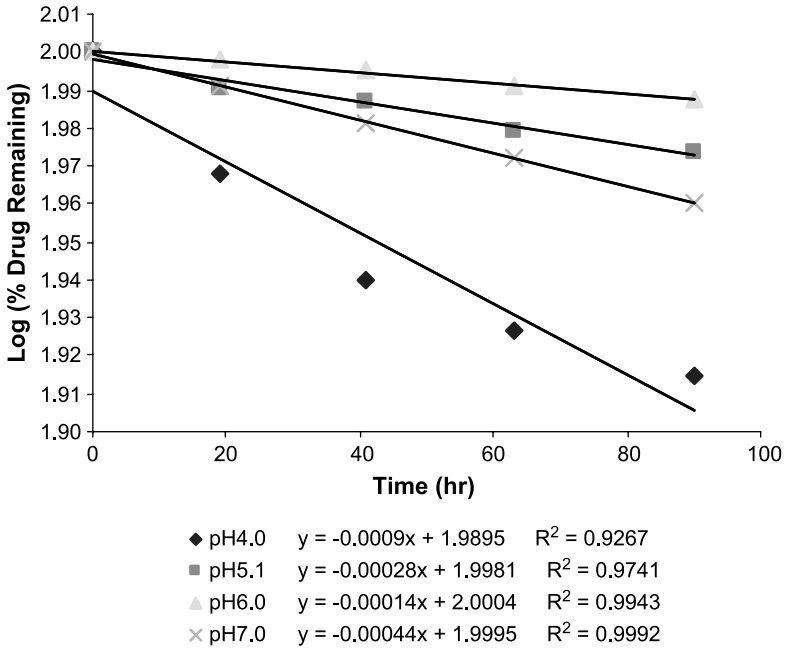

Figure 2. Typical degradation profiles (CT54004 at pH 4.0, 5.1, 6.0, and 7.0).

Table 3. Estimated degradation rates at investigated pH of MLN1021.

	Degradation rate	
pH of Drug solution	k (hr^{-1})	log k
1.1	0.0238	−1.62
3.0	0.0045	−2.35
4.1	0.0012	−2.92
4.8	0.0004	−3.40
5.2	0.0003	−3.52
5.8	0.00012	−3.92
6.1	**0.00010**	**−4.00**
6.6	0.00012	−3.92
7.1	0.0002	−3.70
8.1	0.0043	−2.37
9.8	0.0296	−1.53

The bold font indicates the most stable condition.

and CT54004, respectively. The slope of the line was used as an approximation of the pseudo-first order reaction rate to compare the stability of the compounds under each pH. Summarized in Tables 3 and 4 are the pseudo-first order degradation rates of MLN1021 and CT54004 under the tested pH, respectively. It was found that the degradation rate was lowest at pH 6.1 for MLN1021 and at pH 6.0 for CT54004, respectively. This was consistent to the findings on % drug remaining.

The pH-rate profile was U-shaped as shown in Fig. 3. Both compounds were quite stable in the middle range of pH. They were least stable at the two extreme ends of pH (pH<3 and >8). The optimum pH for stability of MLN1021 and CT54004 was around 6.1 and 6.0 from experimental point of view, respectively.

Theoretical Prediction of Optimum pH

The optimum pH for both compounds was predicted based on the pH-degradation rate profiles. The data was presented as the plot of log k against pH in Figs. 4 and 5, assuming the kinetic model is a pseudo first order. Both profiles are defined V-shapes.

For MLN1021, the slopes of the straight-line segments at low and high pH are −0.4912 and 0.7823, respectively, and the intercepts of the two straight-line segments are −0.9905 and −9.0592, respectively. Taking these data into Eq. 6, the optimum pH of 6.18 for MLN1021 was obtained. This value is very close to the experimental data of pH 6.1.

For CT54004, the slopes of the straight-line segments at low and high pH are −0.4394 and 0.5143, respectively, and the intercepts of the two straight-line segments are −1.2837 and −6.9935, respectively. Taking these data into Eq. 6, the optimum pH of 5.92 for CT54004 was obtained. This value is very close to the experimental data of pH 6.0.

Considering both slopes were not close to 1 or −1, the mechanism may not be taken as specific-acid-basic-catalyzed reactions. Some other possible factors may be involved in this reaction. Other data from our previous oxidative stress stability study at different temperatures support the assumption that oxidative reaction may involve in the process (data not shown). This could also be further elucidated by identifying the structure of the degradants. The larger slope in higher pH than lower pH suggests that this reaction is more sensitive to high pH. The information hints us the lower pH in formulation will be more beneficial.

The optimum pHs for both compounds are very close. This might be due to their similar chemical structures. The only difference in the structures of the two compounds is the substituted functional groups on benzene: $-OCH_3$ for MLN1021 and −Cl for CT54004.

Ideal Experimental Design

Based on the experience of this study, an ideal experimental design is proposed as following,

Table 4. Estimated degradation rates at investigated pH of CT54004.

	Degradation rate	
pH of Drug solution	k (hr^{-1})	log k
3.2	0.0054	−2.268
4.0	0.0009	−3.046
4.7	0.00049	−3.310
5.1*	0.00029	−3.538
5.1	0.00028	−3.553
5.4	0.00021	−3.678
5.7	0.00017	−3.770
5.8	0.00015	−3.824
6.0*	**0.00014**	**−3.854**
6.0	**0.00014**	**−3.854**
6.3	0.00016	−3.796
6.5	0.00020	−3.699
7.0	0.00044	−3.357
8.1	0.0029	−2.538

The bold font indicates the most stable condition.
*These treatments were repeated in the follow-up study.

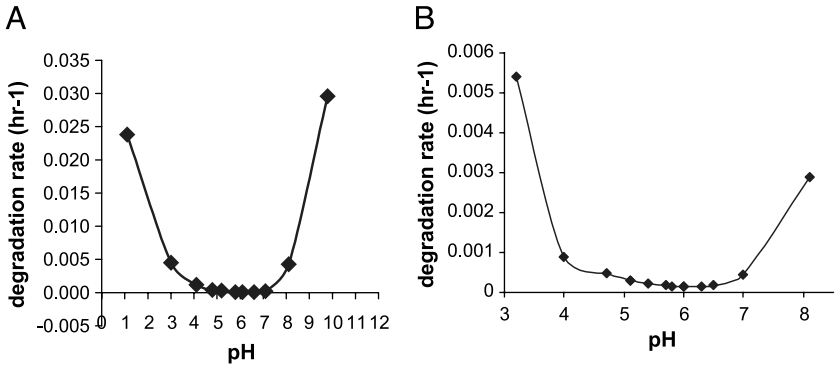

Figure 3. The pH-degradation rate profiles of (A) MLN1021 and (B) CT54004 at 60°C.

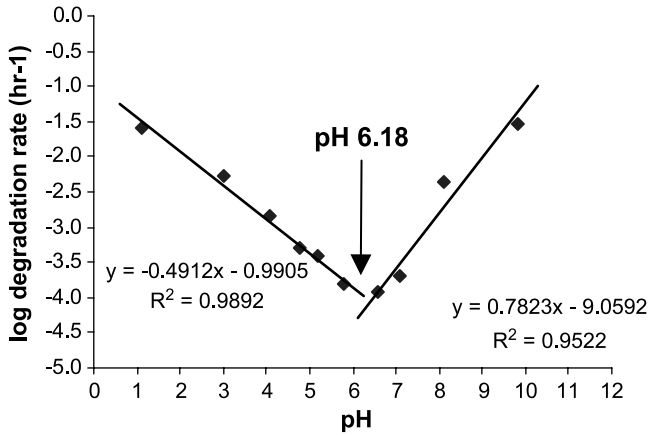

Figure 4. Prediction of the optimum pH of MLN1021 solution based on the pH-degradation rate profile.

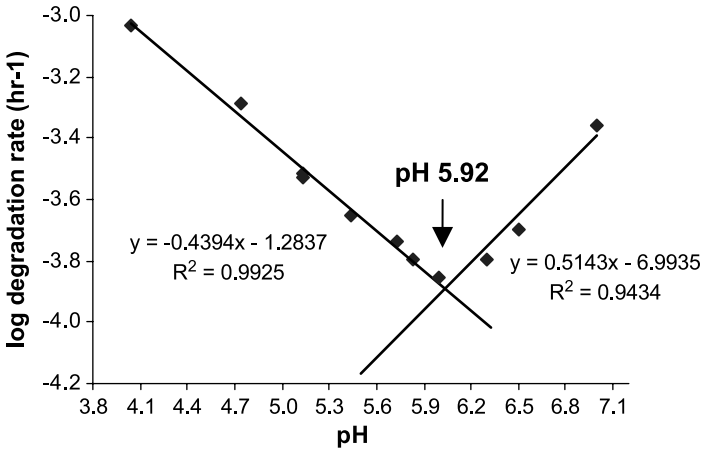

Figure 5. Prediction of the optimum pH of CT54004 solution based on the pH-degradation rate profile.

which will be useful for future study on similar drug candidate:

1. Investigate three pHs at extreme low pH and three pHs at extreme high pH.
2. Plot the % drug remaining versus time, and get psudo-first order degradation rate.
3. Plot the pH-degradation profile (semilog plot, confirm if it's V-shaped curve).
4. Predict the theoretical optimum pH by using the equation for pH_{min} (Eq. 6).
5. Confirm the pH_{min} by experiment. Investigate three pHs: pH_{min} and $pH_{min} \pm 0.3$.

This design only requires investigating 9 pHs totally, and it takes much less time since the degradation at extreme pH is faster. Comparing to the traditional labor intensive procedure, this design liberate the investigator without scarifying the quality. The investigation at extreme pH will also generate more degradation, which is beneficial to less error in calculation of rate constant. In addition, fine tune investigation around predicted optimum pH is more aimful and accurate.

CONCLUSIONS

MLN1021 and CT54004 are quite stable in the middle pH range, and least stable at pH<3 and >8. The optimum pH for drug stability is at about 6.1 (MLN1021) and 6.0 (CT54004), which are consistent to the theoretical prediction of 6.18 and 5.92. The mechanism of the reaction is not specific-acid/base-catalyzed reactions. The proposed experimental design will be useful for future study on similar drug candidate.

REFERENCES

1. Carstensen, J.T. *Drug Stability*; Marcel Dekker: New York, 1995.
2. Connors, K.A.; Amidon, G.L.; Stella, V.J. *Chemical Stability of Pharmaceuticals*; John Wiley & Sons: New York, 1986.
3. Zhang, P.; Bao, L.; Zuckett, J.F.; Goldman, E.A.; Jia, Z.J.; Arfsten, A.; Edwards, S.; Sinha, V.; Hutchaleelaha, A.; Park, G.; Lambing, J.L.; Hollenbach, S.J.; Scarborough, R.M.; Zhu, B. Design, synthesis, and SAR of anthranilamide-based factor Xa inhibitors incorporating substituted biphenyl P4 motifs. Bioorg. Med. Chem. Lett. **2004**, *14*, 983–987.
4. *Geigy Scientific Table*. 7th Ed.; Medical Education Division, Ciba-Geigy Corp.: Ardsley, New York, 1974.
5. McIlvaine, T.C. A buffer solution for colorimetric comparison. J. Biol. Chem. **1921**, *49*, 183–186.