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Effect of Ionic Strength on Solution Stability of PNU-67590A, A Micellar Prodrug of Methylprednisolone

Hirokazu Okamoto,[1,2] Kiyoko Mori,[1] Kumiko Ohtsuka,[1] Hiroyuki Ohuchi,[1] and Hiroaki Ishii[1]

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Purpose. PNU-67590A is a water-soluble micellar prodrug of methylprednisolone (MP). The major products of degradation of PNU-67590A are MP by hydrolysis and methylprednisolone 17-suleptanate (17-E) by 21 → 17 acyl migration. The effect of ionic strength on micelle formation and stability of PNU-67590A in aqueous solution was examined.

Methods. PNU-67590A solutions at pH 2 and 8 and ionic strength of 0.05, 0.1, 0.2, and 0.4 M were maintained at 25°C in the dark to measure MP and 17-E levels over time.

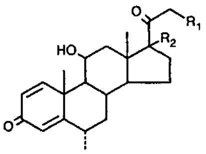
Results. The rate of degradation of micellar PNU-67590A at pH 8 was less than that of monomeric PNU-67590A, and vice versa at pH 2. Increase in ionic strength decreased both the critical micelle concentration of PNU-67590A and the degradation of micelle PNU-67590A at both pHs, resulting in improved overall stability of PNU-67590A.

Conclusions. Formulation of PNU-67590A in a concentrated solution with high ionic strength will maximize stability and shelf-life.

KEY WORDS: stability; ionic strength; pH; critical micelle concentration; self-micellization.

INTRODUCTION

PNU-67590A (previously U-67590A; methylprednisolone suleptanate; sodium(+)−11β,17-dihydroxy-6α-methyl-21-[[8-[methyl(2-sulfoethyl)amino]-1,8-dioxooctyl]oxy]-1,4-pregnadiene-3,20-dione) is a water-soluble micellar prodrug of methylprednisolone (MP) which is relatively stable in water compared with other prodrugs such as methylprednisolone hemisuccinate (1). The sulfonate group in the pro-moiety dissociates in solutions at a relatively low pH to make the PNU-67590 anion. The major products of degradation of PNU-67590A are MP by hydrolysis in acidic or neutral to alkaline solution and methylprednisolone 17-suleptanate (17-E) by 21 → 17 acyl migration in neutral to alkaline solution. The pH-hydrolysis rate profile of PNU-67590A has the typical V-shaped curve due to contributions from acid-catalyzed, base-catalyzed, and neutral reactions (2). The effect of self-micellization on aqueous stability of PNU-67590A was studied by Anderson *et al* (2). The pH-rate minimum (pH_{min}) of a dilute solution was around pH 4.8. PNU-67590A in concentrated solution forms micelles, the effect of which is to shift the pH_{min} toward a higher pH and to lower the hydrolysis rate at pH_{min} compared to that of a dilute solution due to acceleration of acid-catalyzed hydrolysis and inhibition of base-catalyzed hydrolysis.



Compound	R_1	R_2
PNU-67590A	$-OCO(CH_2)_8CON(CH_3)CH_2CH_2SO_3Na$	$-OH$
MP	$-OH$	$-OH$
17-E	$-OH$	$-OCO(CH_2)_6CON(CH_2)CH_2CH_2SO_3Na$

It is known that the ionic strength of solution affects the rate of degradation of electrolytes and the critical micelle concentration (cmc) of surfactants. Our previous study showed that the rates of hydrolysis and 21 → 17 acyl migration of PNU-67590A in a dilute solution containing monomeric PNU-67590A were increased with increase in ionic strength (3). Changing the ionic strength of solution may affect the stability of drugs forming micelles and the cmc of micellar drugs as well as the stability of monomeric drugs, which may in turn result in variation in the overall stability of the solution.

In this study, the stability of PNU-67590A at various concentrations and at various ionic strengths was evaluated at pH 2 and pH 8 to examine the effect of ionic strength on micelle formation and stability of PNU-67590A in aqueous solution.

MATERIALS AND METHODS

Materials

PNU-67590A bulk drug lot (A2)7231-GR-155-J038 was supplied by The Upjohn Company, MI. Trisodium citrate dihydrate, sodium chloride, acetic acid, sodium acetate, ammonium acetate, acetonitrile, tetrahydrofuran, Bis-Tris (bis(2-hydroxyethyl)iminotris(hydroxymethyl)methane), and 1 mol/L hydrochloric acid were purchased from Wako Pure Chemical Industries, Ltd., Osaka. Trizma® base (Tris; tris(hydroxymethyl)aminomethane) was purchased from Sigma Chemical Co., MO. Deionized water was prepared with the Milli-Q SP TOC (Japan Millipore Ltd., Tokyo).

Preparation of Stability Solutions

The compositions of PNU-67590A solutions (0.1 mg/mL through 20 mg/mL) at pHs 8 (Tris buffer) and 2 (hydrochloric acid) and ionic strengths of 0.05, 0.1, 0.2 and 0.4 M were calculated using a computer program we developed (4) and prepared by mixing several stock solutions listed in Table I in 10 mL volumetric flasks. A portion of sodium chloride stock solution was replaced by an appropriate amount of the PNU-67590A bulk drug to maintain the ionic strength on the assumption that PNU-67590A dissociated completely into PNU-67590 anions and sodium cations.

Stability Study

As soon as the PNU-67590A solution had been prepared, the flask containing it was immersed in a water bath at 25°C in the dark and the initial sample was removed. Samples were taken at 1, 2, and 3 hours at pH 8 and at 1.5, 3, and 4.5 hours at pH 2. Degradation of PNU-67590A was stopped by mixing the pH 8 sample with the same volume of 0.2 M acetate buffer (pH 4.6) and by mixing the pH 2 sample with the same volume of 0.01 M trisodium citrate solution (pH 8.9). The areas corresponding to MP and 17-E on chromatograms were plotted against

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[1] Pharmacia & Upjohn, Tsukuba Research Laboratories, 23 Wadai, Tsukuba, Ibaraki 300-42, Japan.

[2] To whom correspondence should be addressed. (e-mail address: hirokazu.okamoto@am.pnu.com)

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Table I. Compositions of Stability Solutions (10-mL) with X mg/mL PNU-67590A

Ionic strength, M	Stock solution volume (mL) to prepare 10 mL of pH 8 solution			Stock solution volume (mL) to prepare 10 mL of pH 2 solution	
	0.1 M Tris	0.3114 M HCl	1 M NaCl[a]	0.05 M HCl	1 M NaCl[a]
0.05	5.00	0.943	0.206-X/67.38	2.42	0.379-X/67.38
0.10	5.00	0.969	0.698-X/67.38	2.47	0.876-X/67.38
0.20	5.00	1.00	1.69-X/67.38	2.45	1.88-X/67.38
0.40	5.00	1.03	3.68-X/67.38	2.18	3.89-X/67.38

[a] When preparing X mg/mL PNU-67590A solution, the volume of sodium chloride stock solution was decreased by X/67.38 mL to adjust the ionic strength on the assumption that equivalent moles of sodium chloride and PNU-67590A had the same effect on ionic strength.

time to obtain the slope of the regression line. When the regression coefficient was larger than 0.95, the slope was divided by the initial area of PNU-67590A on the chromatogram to obtain the apparent first-order overall hydrolysis rate constant ($k1_{obs}$) and acyl migration rate constant ($k3_{obs}$). The solution pHs were measured for the initial and final samples. The means and standard deviations of pHs were 1.93 ± 0.03 and 8.00 ± 0.04, respectively.

The rate constants $k1_{obs}$ and $k3_{obs}$ at 25°C in the dark were determined for 0.18 mg/mL solutions at pHs 1 and 2 (hydrochloric acid), 3, 4, and 5 (acetate buffer), 6 and 7 (bis-Tris buffer), and 8 and 9 (Tris buffer) to determine pH-rate profiles.

Assay Procedures

PNU-67590A, MP, and 17-E were measured using a high performance liquid chromatography (HPLC) system (Shimadzu Co., Kyoto), composed of a system controller (SCL-10A), pump (LC-10AD), autoinjector (SIL-10A), column oven (CTO-10A), UV spectrophotometric detector (SPD-10A), and integrator (C-R4A). The mobile phase was 750 mL water containing 11.6 g of ammonium acetate and acetic acid q.s. (quantum sufficient) (pH 5.8), 210 mL of acetonitrile, and 40 mL of tetrahydrofuran at a flow rate of 1.6 mL/min. The column was an Inertsil Ph (4.6 × 150 mm, 5 μm) (GL Science, Tokyo, Japan) heated at 40°C. Ultraviolet absorption was measured at 254 nm. The injection volume was 15 μL.

RESULTS

Change in the rate of hydrolysis or acyl migration with PNU-67590A concentration was analyzed using a phase-separation model (5). Below the cmc, the system contains monomers dispersed in water. Above the cmc, the system contains two phases, one consisting of monomers in water and the other represented by micelles. The monomer concentration, C_{mon}, is constant and equal to the cmc at or above the cmc, but equal to the total PNU-67590A concentration, C_t, below the cmc. The overall rate of hydrolysis or acyl migration is considered to be the summation of rates of hydrolysis or acyl migration for monomeric and micellar PNU-67590A:

$$k_{obs}C_t = k_{mon}C_{mon} + k_{agg}(C_t - C_{mon}) \qquad (1)$$

where k_{obs}, k_{mon}, and k_{agg} are the overall rate constant, rate constant for monomeric PNU-67590A, and rate constant for micellar PNU-67590A, respectively. Rearrangement of Eq. (1) yields the relationship between k_{obs} and C_t above the cmc:

$$k_{obs} = k_{agg} + (k_{mon} - k_{agg})\mathrm{cmc}/C_t \qquad (2)$$

and below the cmc:

$$k_{obs} = k_{mon} \qquad (3)$$

The plot of k_{obs} against $1/C_t$ initially yields a line with slope of $(k_{mon} - k_{agg})$cmc, up to 1/cmc. The k_{agg} value is determined as the y-intercept of the line, and cmc is calculated as (slope)/$(k_{mon} - k_{agg})$. When C_t is smaller than the cmc, the k_{obs} will be independent of C_t and equal to k_{mon}.

Figures 1(A) and 1(B) show the relationship between hydrolysis or acyl migration rate constant at pH 8 and $1/C_t$. Both the overall hydrolysis rate constant ($k1_{obs,pH8}$) and acyl migration rate constant ($k3_{obs,pH8}$) increased linearly with $1/C_t$ up to C_t = cmc. When $1/C_t > 1/\mathrm{cmc}$, $k1_{obs,pH8}$ and $k3_{obs,pH8}$ were almost constant. At pH 2, the overall hydrolysis rate constant ($k1_{obs,pH2}$) decreased with $1/C_t$ up to C_t = cmc (Figure 1(C)). The overall rate constants in a concentrated PNU-67590A solution at both pHs decreased with increase in ionic strength.

The rate constants for monomeric and micellar PNU-67590A and the cmc value calculated using the phase-separation model are listed in Table II. The rate constant, k, is related to the solution ionic strength (I) as follows (6):

$$\log k = \log k_0 + 2QZ_aZ_bI^{0.5}/(1 + I^{0.5}) \qquad (4)$$

where k_0 is the rate constant at I = 0, Z_a and Z_b are the charges of reactants, and 2Q = 1.018 for aqueous solution at 25°C. This equation holds up to an ionic strength of about 0.1 M, but is often applied at higher ionic strengths as well. The calculated rate constants are plotted against $I^{0.5}/(1 + I^{0.5})$ in Figures 2(A), 2(B), and 2(C). The hydrolysis rate constant ($k1_{agg,pH8}$) and acyl migration rate constant ($k3_{agg,pH8}$) for micellar PNU-67590A at pH 8 were smaller than those ($k1_{mon,pH8}$ and $k3_{mon,pH8}$) for monomeric PNU-67590A. The values of $k1_{agg,pH8}$ and $k3_{agg,pH8}$ decreased with ionic strength, while the values of $k1_{mon,pH8}$ and $k3_{mon,pH8}$ increased with it. At I = 0.4 M, $k1_{agg,pH8}$ and $k3_{agg,pH8}$ were 30 and 18 times smaller than $k1_{mon,pH8}$ and $k3_{mon,pH8}$, respectively. However the hydrolysis rate constant ($k1_{agg,pH2}$) for micellar PNU-67590A at pH2 was larger than that for monomeric PNU-67590A ($k1_{mon,pH2}$). The values of $k1_{agg,pH2}$ and $k1_{mon,pH2}$ grew closer with increase in ionic strength. The calculated cmc value decreased with ionic strength at both pHs (Table II).

DISCUSSION

The compositions of the tested solutions were calculated using a computer program (4) on the assumption that the alkyl-

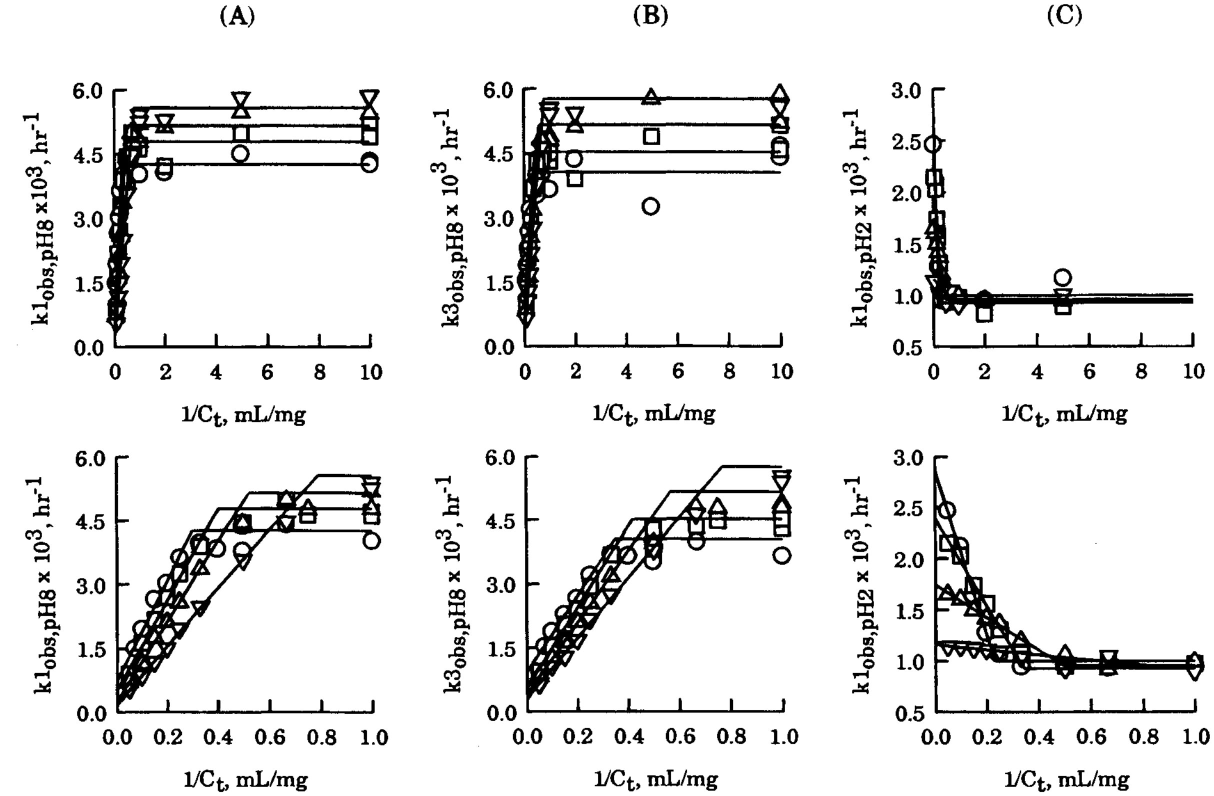

Fig. 1. Overall hydrolysis rate constant at pH 8 (A), overall acyl migration rate constant at pH 8 (B), and overall hydrolysis rate constant at pH 2 (C) as functions of PNU-67590A concentration. Ionic strengths are adjusted to 0.05 M (○), 0.10 M (□), 0.20 M (△), and 0.40 M (▽). Lines were calculated using Eqs. (2) and (3). Upper figures show all data obtained, while lower figures show data for 1/Ct ≤ 1 mL/mg (Ct ≥ 1 mg/mL).

Table II. Summary of Hydrolysis Rates, Acyl Migration Rates, and Critical Micelle Concentrations

Degradation pathway	Parameter	Ionic strength, M			
		0.05	0.10	0.20	0.40
Hydrolysis at pH 8	$k1_{mon,pH8} \times 10^3$, hr^{-1a}	4.26	4.78	5.16	5.57
	$k1_{agg,pH8} \times 10^3$, hr^{-1b}	0.749	0.514	0.262	0.187
	cmc, mg/mL	3.32	2.47	1.91	1.26
Acyl Migration at pH 8	$k3_{mon,pH8} \times 10^3$, hr^{-1c}	4.05	4.52	5.16	5.76
	$k3_{agg,pH8} \times 10^3$, hr^{-1d}	0.921	0.550	0.409	0.314
	cmc, mg/mL	2.88	2.39	1.77	1.30
	$k1_{mon,pH2} \times 10^3$, hr^{-1e}	0.996	0.924	0.954	0.951
Hydrolysis at pH 2	$k1_{mon,pH2} \times 10^3$, hr^{-1f}	2.88	2.40	1.75	1.16
	cmc, mg/mL	4.27	2.90	2.01	1.21

[a] $k1_{mon,pH8}$ determined with $C_t \leq 1.5$ mg/mL, $C_t \leq 1.5$ mg/mL, $C_t \leq 1.5$ mh/mL, and $C_t \leq 1.0$ mg/mL at ionic strengths 0.05, 0.10, 0.20, and 0.40 M, respectively.

[b] $k1_{agg,pH8}$ determined with $C_t \geq 4.0$ mg/mL, $C_t \geq 3.0$ mg/mL, $C_t \geq 3.0$ mg/mL, $C_t \geq 2.0$ mg/mL at ionic strengths 0.05, 0.10, 0.20, and 0.40 M, respectively.

[c] $k3_{mon,pH8}$ determined with $C_t \leq 1.5$ mg/mL, $C_t \leq 1.5$ mg/mL, $C_t \leq 1.5$ mh/mL, and $C_t \leq 1.0$ mg/mL at ionic strengths 0.05, 0.10, 0.20, and 0.40 M, respectively.

[d] $k3_{agg,pH8}$ determined with $C_t \geq 4.0$ mg/mL, $C_t \geq 3.0$ mg/mL, $C_t \geq 3.0$ mg/mL, $C_t \geq 2.0$ mg/mL at ionic strengths 0.05, 0.10, 0.20, and 0.40 M, respectively.

[e] $k1_{mon,pH2}$ determined with $C_t \leq 1.5$ mg/mL, $C_t \leq 1.5$ mg/mL, $C_t \leq 1.5$ mg/mL, $C_t \leq 1.0$ mg/mL at ionic strengths 0.05, 0.10, 0.20, and 0.40 M, respectively.

[f] $k1_{agg,pH2}$ determined with $C_t \geq 5.0$ mg/mL, $C_t \geq 4.0$ mg/ml, $C_t \geq 3.0$ mg/mL, and $C_t \geq 4.0$ mg/mL, at ionic strengths 0.05, 0.10, 0.20, and 0.40 M, respectively.

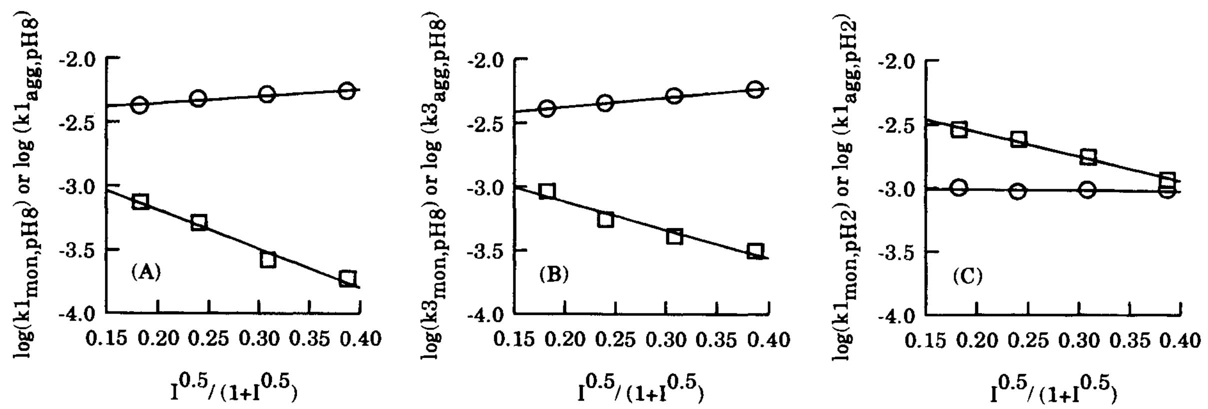

Fig. 2. Hydrolysis rate constants for monomeric (○) and micellar (☐) PNU-67590A at pH 8 (A), acyl migration rate constants for monomeric (○) and micellar (☐) PNU-67590A at pH 8 (B), and hydrolysis rate constants for monomeric (○) and micellar (☐) PNU-67590A at pH 2 (C) as functions of ionic strength. The lines are regression lines calculated from 4 data points each. Rate constants are given in units of hr^{-1}.

sulfonate group of PNU-67590 is completely dissociated even at pH 2, i. e., its pKa value is much smaller than 2. This assumption appears to be valid, since the actual pH values of the pH 2 Solutions (Table I) were close to 2.0 (1.93 ± 0.03). If the pKa value had been near or larger than 2, the measured pH values of the pH 2 Solutions would have been higher than 2. For instance, theoretical calculations performed with the program showed that the pH of the 20 mg/mL solution would be 2.4 if the pKa value were 2.0.

The pH rate profile of hydrolysis of PNU-67590A has a slope of about -1 in acidic conditions and a slope of about 1 in neutral to alkaline conditions, suggesting that hydrolysis is acid-catalyzed at low pH and base-catalyzed at high pH. The 21 $\rightarrow$ 17 acyl migration is practically unobserved at low pH but is base-catalyzed at high pH (Figure 3). Eq. (4) predicts that the hydrolysis and acyl migration rate constants will be increased at pH 8 (reactants are PNU-67590 anion and hydroxy ion) but decreased at pH 2 (reactants are PNU-67590 anion and hydronium ion). This was the case for the rate constants at pH 8 for monomeric PNU-67590A ($k1_{mon,pH8}$ and $k3_{mon,pH8}$), although the slopes in Figures 2(A) and 2(B) were smaller than predicted by Eq. (4). At pH 2, $k1_{mon,pH2}$ was almost constant regardless of ionic strength. One of the reasons for these deviations from theoretically predicted values may be that the ionic strength examined was higher than that at which the equation holds.

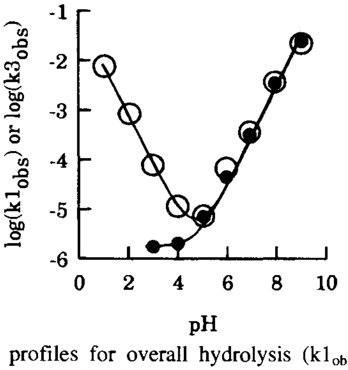

Fig. 3. pH-rate profiles for overall hydrolysis ($k1_{obs}$, ○) and acyl migration ($k3_{obs}$, ●). The rate constants are given in units of hr^{-1}.

The degradation of micellar PNU-67590A at pH 8 decreased with ionic strength, contrary to the prediction obtained with Eq. (4), suggesting a mechanism other than the kinetic salt effect. We examined the extraction of di(2-ethylhexyl) phthalate (DEHP), a lipid soluble plasticizer, by PNU-67590A micelles from a plastic infusion line. The amount of DEHP extracted by a unit weight of PNU-67590A micelles increased with ionic strength, suggesting that PNU-67590A micelles became more lipophilic with increase in ionic strength (unpublished data). The ester linkage in PNU-67590A is located quite far from the charged substituents and near the center of the molecule or the interior of micelles (2). Increase in lipophilicity of the micelles may limit the access of hydroxide ion and hydronium ion to the ester linkage. This mechanism would explain the decrease in $k1_{agg,pH2}$ at pH 2 as well as $k1_{agg,pH8}$ and $k3_{agg,pH8}$ at pH 8 we observed. Although the effect of ionic strength on neutral (water) catalysis was not examined in this study, water-catalyzed degradation may be reduced by increase in ionic strength, supposing that the mechanism discussed above is in effect.

The rate constants ($k1_{agg,pH8}$ and $k3_{agg,pH8}$) for micellar PNU-67590A at pH 8 were smaller than those ($k1_{mon,pH8}$ and $k3_{mon,pH8}$) for monomeric PNU-67590A, while $k1_{agg,pH2}$ was larger than $k1_{mon,pH2}$ at pH2. PNU-67590A dissociates to PNU-67590 anion and sodium cation to form anionic micelles. Hydroxide ion, hydronium ion, and/or water may penetrate to the interior of micelles to attack ester linkages. Negative charge on the surface of micelles may enhance access of hydronium ions at pH 2, but act as a barrier to hydroxide ions at pH 8.

PNU-67590A is formulated in 90 mg/mL and 180 mg/mL solutions. Table III compares some hydrolysis rate constants for examination of the effect of PNU-67590A concentration and ionic strength on the hydrolysis of PNU-67590A. At pH 8 (base-catalyzed condition), the hydrolysis rate constant reduces with concentration and with ionic strength when $C_t \geq$ cmc. The 180 mg/mL solution at I = 0.4 M is 19 times more stable than the 1 mg/mL solution at I = 0.05. At pH 2 (acid-catalyzed condition), the hydrolysis rate constant increases with concentration but decreases with ionic strength. The stability of the 180 mg/mL solution at I = 0.4 M is comparable to that of 1 mg/mL solution at I = 0.05 M. Figure 2(C) suggests that

Table III. Estimates of Overall Hydrolysis Rate Constants from Hydrolysis Rate Constants for Monomeric and Micellar PNU-67590A

		$k1_{obs,pH8} \times 10^3$ or $k1_{obs,pH2} \times 10^3$, hr^{-1}		
pH	Ionic Strength, M	$C_t = 1$ mg/mL[a]	$C_t = 10$ mg/mL[b]	$C_t = 180$ mg/mL[b]
8	0.05	4.26	1.92	0.813[c]
8	0.4	5.57	0.869	0.225
2	0.05	0.996	2.08	2.84[c]
2	0.4	0.951	1.13	1.16

[a] $k1_{obs} = k1_{mon}$.

[b] $k1_{obs} = k1_{agg} + (k1_{mon} - k1_{agg})cmc/C_t$.

[c] The 180 mg/mL solutions cannot be prepared at ionic strengths as low as 0.05 M due to the contribution of PNU-67590A itself to ionic strength. Value for this solution is presented informational purpose only.

$k1_{agg,pH2}$ will be smaller than $k1_{mon,pH2}$ at ionic strengths above 0.4 M and will minimize overall hydrolysis of concentrated PNU-67590A solution at pH 2. When formulating PNU-67590A at pH_{min}, acid-catalyzed and base-catalyzed degradations as well as water-catalyzed degradation occur. Formulating PNU-67590A in a concentrated solution with high ionic strength will minimize all these degradation reactions and maximize stability and shelf-life. Ionic strength can in general be increased within the limits of physiological osmolarity. However, if an injectable such as PNU-67590A sterile solution is administered after dilution with a large volume of intravenous fluid such as normal saline, the ionic strength of the injectable may be increased above physiological osmolarity.

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