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Research Article

Potential Improvement in Shelf Life Using the Prodrug Approach. II. A Systematic Examination of Kinetic Requirements

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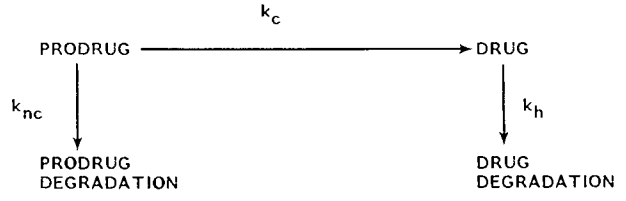
The utilization time (UT) for a solution of a prodrug that is rapidly and completely converted to drug in the blood may be longer than the time for 10% loss of the initial concentration. The UT for an *intravenous* prodrug solution is the period during which the *total* prodrug and drug concentration exceeds 90% of the initial concentration. The influence of the rate of prodrug degradation (k_{nc}), its conversion (k_c) to drug, and the subsequent drug degradation (k_h) on the UT of a stored solution was examined by simulating the prodrug and drug concentration–time courses. The ratio of the shelf life of a prodrug solution to that of the parent drug (UT_{ratio}) was calculated using a wide range of values for the three rate constants. Three-dimensional plots relating the UT_{ratio} to the k_c, k_{nc}, and k_h values provide a basis for making *a priori* assessments of kinetic requirements for designing a prodrug to increase storage time. A parenteral prodrug intended to increase storage time may have a larger overall rate of loss than the parent drug, but it must have a smaller degradation rate ($k_{nc} < k_h$) to be successful. The UT for an *oral* prodrug solution depends upon the bioavailability of the prodrug relative to the drug in addition to the values for k_{nc}, k_c, and k_h. Two ampicillin prodrugs were used as models to calculate actual UT_{ratio} versus pH profiles. Intravenous solutions showed modest gains in the UT_{ratio} in the acid region, whereas oral solutions reached a UT_{ratio} as high as 22 by combining favorable rate constants with increased bioavailability. These actual UT_{ratio} versus pH profiles were interpreted in terms of the theory established using the simulations.

KEY WORDS: prodrugs; shelf life; parenteral; oral; utilization time; computer simulation; bacampicillin; talampicillin; ampicillin; stability; storage; prodrug design.

INTRODUCTION

Prodrugs are inactive drug precursors formed by covalently bonding a drug to an inert chemical by a linkage that may be broken (by any mechanism) to yield the drug *in vivo* (1,2). However, a prodrug in solution may prematurely convert to the drug via chemical hydrolysis during storage. It may also form biologically inactive products via a degradation pathway similar to that for the parent drug. For example, hydrolysis of ester prodrugs of penicillins may yield the corresponding penicillin while simultaneously forming inactive β-lactam degradation products. These rate processes are represented by



Scheme I

where k_c is the first-order rate constant for prodrug conversion to drug; k_{nc} and k_h are the prodrug and drug degradation constants for formation of inactive products.

This investigation determined what rate constant values are required to produce a prodrug solution having a longer shelf life than the parent drug. A solution of the parent drug was presumed useful until its concentration decreased to 90% of its initial value (T_{90}). Prodrug utilization time (UT) was defined as the time during which administration of a prodrug solution provided a bioavailable dose of drug equal to or better than the parent drug at its T_{90}. Two assumptions are implicit throughout this report. Only prodrugs that are rapidly and completely converted to drug in the blood are considered. An extravascular prodrug dose is assumed to produce a drug plasma concentration–time course similar in shape to that of the parent drug but that may differ by a concentration scaling factor so that the bioavailable fraction can vary. Under these conditions, a fresh *intravenous* prodrug solution is bioequivalent to the parent drug. Moreover, the bioavailable dose remaining at any time is equal to the *total* concentration of the prodrug plus the drug. The UT for this solution is the time during which this total concentration exceeds 90% of the initial prodrug concentration. This UT is influenced by the three rate processes that control the concentration of prodrug and drug in Scheme I.

The UT for an extravascular prodrug solution is influenced not only by the processes occurring during storage

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but also by the bioavailability of the prodrug relative to the drug. Unlike the intravenous case, extravascular doses may not be bioequivalent. Consequently, the UT for an *oral* prodrug solution may differ from the UT for its intravenous solution as shown in this report using ampicillin prodrugs.

The specific aims of this research were

(i) to calculate intravenous UT as a function of k_c, k_{nc}, and k_h values by using computer simulations of Scheme I;
(ii) to find which, if any, rate constant values provide a UT that exceeds the T_{90} value for the parent drug;
(iii) to provide a basis for making *a priori* assessments regarding the k_c and k_{nc} values required for a favorable UT/T_{90} ratio when the k_h values for the drug are known;
(iv) to use previously reported pH–rate expressions for k_h, k_c, and k_{nc} (3), together with oral bioavailability values, to create examples of actual UT/T_{90} versus pH profiles for intravenous and oral prodrug solutions; and
(v) to establish the relationship between these actual UT/T_{90} versus pH profiles and the rate constants that were found by computer to result in a prodrug storage advantage.

THEORY

Predicted Rate Constant Requirements for Prodrug Storage Advantage. In Scheme I, the concentration of prodrug [PD] and drug [D] as a function of time may be written

$$[PD]_t = [PD]_0 e^{-(k_{nc}+k_c)t} \quad (1)$$

$$[D]_t = \frac{[PD]_o k_c}{[k_h - (k_{nc} + k_c)]} [e^{-(k_{nc}+k_c)t} - e^{-(k_h)t}] \quad (2)$$

where $[PD]_0$ is the initial prodrug concentration (2). First-order loss of the parent drug may be described by

$$[D]_t = [D]_0 e^{-(k_h)t} \quad (3)$$

where $[D]_0$ is the initial concentration. The fraction, f_1, remaining in a *drug* solution as a function of time is described by rearranging Eq. (3) to give

$$f_1 = \frac{[D]_t}{[D]_0} = e^{-(k_h)t} \quad (4)$$

For an *intravenous* solution of prodrug, the bioavailable dose remaining as a function of time is the sum of the prodrug and drug. The fraction remaining as a function of time, f_2, is therefore the sum of Eqs. (1) and (2) divided by the initial prodrug concentration:

$$f_2 = \frac{[PD]_t + [D]_t}{[PD]_0} = \frac{1}{[k_h - (k_{nc} + k_c)]} [(k_h - k_{nc})e^{-(k_{nc}+k_c)t} - k_c e^{-(k_h)t}] \quad (5)$$

The difference between the fraction remaining in a prodrug solution and that in a drug solution as a function of time is represented by

$$f_2 - f_1 = \frac{k_h - k_{nc}}{[k_h - (k_{nc} + k_c)]} [e^{-(k_{nc}+k_c)t} - e^{-(k_h)t}] \quad (6)$$

The prodrug will provide a longer storage time than the drug for any combination of k_c, k_{nc}, and k_h values that imparts a positive value to Eq. (6), i.e., $f_2 > f_1$.

Case 1: Favors the Drug. When the drug is more stable to degradation than the prodrug ($k_h < k_{nc}$), Eq. (6) is negative ($f_2 < f_1$). Thus, a solution of the drug will provide a longer shelf life than a solution of the prodrug.

Case 2: Favors the Prodrug. When the prodrug is more stable to degradation than the drug ($k_{nc} < k_h$), Eq. (6) is positive ($f_2 > f_1$) and a prodrug solution will provide a longer storage time than a drug solution. The rate constant for overall loss of the prodrug ($k_{sum} = k_{nc} + k_c$) may exceed that of the drug (k_h) but Eq. (6) remains positive so long as $k_{nc} < k_h$. Thus, the overall loss of prodrug may be greater than that of the drug, while the prodrug solution still provides a longer storage life than the drug solution.

In summary, Eq. (6) predicts an increase in the shelf life of a prodrug solution except when prodrug degradation (k_{nc}) is more rapid than drug degradation (k_h). Computer simulations were used to measure quantitatively the influence of the k_c, k_{nc}, and k_h values on the prodrug storage stability.

Utilization Time. The shelf life of a drug solution was defined as $T_{90} = 0.105/k_h$. The UT of an *intravenous* prodrug solution was defined as the time at which the *total* prodrug and drug concentration became equal to 90% of $[PD]_0$. The utilization time ratio (UT_{ratio}) was defined as the ratio of the prodrug UT to the drug T_{90} under identical storage conditions, i.e., $UT_{ratio} = UT/T_{90}$.

Whereas UT is a function of the absolute values for k_{nc}, k_c, and k_h, UT_{ratio} is governed only by the relative values of these three rate constants. The values for each rate constant were therefore varied through a range that was sufficient to establish the upper and lower limits of the UT_{ratio} as well as the intermediate values. The concentration–time courses for prodrug, Eq. (1), and drug, Eq. (2), were generated by computer using k_{nc}, k_c, and k_h values (excluding $k_h = 0$, $k_{sum} = 0$, and $k_h = k_{sum}$) systematically combined to define the UT_{ratio} trends adequately. The *total* prodrug and drug in a stored solution was obtained as a function of time by adding the concentrations, $[PD]_t$ and $[D]_t$.

For each set of rate constant values, the *total* concentration of prodrug and drug was evaluated as a function of time to determine reiteratively the time at which this sum reached 90.0 ± 0.01% of the initial prodrug concentration. The UT_{ratio} was calculated for each set of k_h, k_{nc}, and k_c values. Because a wide range of values was employed for each of the three rate constants, a wide range of UT_{ratio} values resulted.

In order to generalize the results, the UT_{ratio} values were incorporated into three-dimensional plots as a function of the two prodrug rate constants, which were normalized with respect to k_h. Thus, for a known value of k_h, the k_{nc} and k_c values associated with a given UT_{ratio} can be obtained from the three-dimensional plots. The interpretation and use of these plots are discussed next.

The Influence of the Conversion and Degradation Rates. The prodrug solution increased the storage time whenever the UT_{ratio} exceeded one. Figure 1 shows the UT_{ratio} values obtained when k_{nc} was less than or equal to k_h ($k_{nc}/k_h \leq 1$) and k_c values were 0 to 10 times k_h. Figure 2 is an enlarged presentation of the region where $k_{nc}/k_h \leq 0.2$ and $k_c/k_h \leq 5.0$. As shown in Fig. 1, the UT_{ratio} was 1.0, independent of the k_c value, when the degradation rates of the prodrug and drug were equal ($k_{nc} = k_h$).

Figures 1 and 2 show that the UT_{ratio} depends primarily on the prodrug degradation rate constant (k_{nc}) relative to the

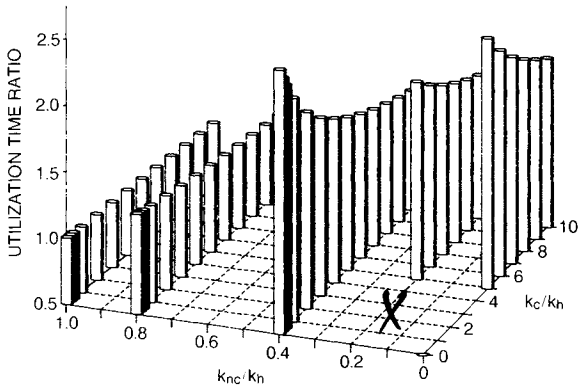

Fig. 1. The utilization time ratio comparing the shelf life (UT) for an *intravenous* solution of a prodrug described by Scheme I to the T_{90} for a solution of the parent drug ($UT_{ratio} = UT/T_{90}$). When $k_{nc} \leq k_h$, the UT_{ratio} is equal to or greater than one and the UT_{ratio} is reduced by increasing k_c. Larger values for the UT_{ratio}, associated with section X, are shown in Fig. 2.

degradation constant for the parent drug (k_h) and to a lesser extent on the prodrug conversion constant (k_c). As predicted in Case 2, the shelf life of a prodrug solution exceeded the drug ($UT_{ratio} > 1$) whenever the prodrug was more stable to degradation ($k_{nc} < k_h$). These UT_{ratio} values were reduced as k_c was enlarged since this increased prodrug conversion to the less stable drug.

These figures can be used to predict the rate constant values necessary to achieve a desired UT_{ratio}. Consider, for example, a drug that has a 4-month shelf life and a minimum desired shelf life of 2 years for marketing. The required UT_{ratio} is therefore 6. The necessary rate constant values can be observed in Fig. 2, where two acceptable examples are $k_{nc} = 0.1\ k_h$ with $k_c \leq 0.3\ k_h$ and $k_{nc} = 0.05\ k_h$ with $k_c \leq 0.5\ k_h$. The range of acceptable values can be used to decide *a priori* whether or not it is feasible to obtain the desired UT_{ratio} through bioreversible chemical modification. For example, the observed β-lactam stabilization via penicillin prodrug formation was about threefold (4–6). This represents a k_{nc}/k_h value of 0.33. A k_{nc}/k_h value of 0.33 would provide a maximum UT_{ratio} of 3 at $k_c = 0$, which would decrease as k_c increased. This ratio is not shown in Fig. 1, but the trend would be similar to that shown for $k_{nc}/k_h = 0.4$.

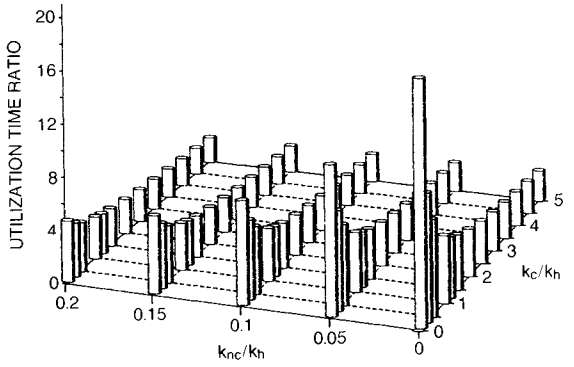

Fig. 2. The UT_{ratio} values taken from section X in Fig. 1. These values represent prodrugs described by Scheme I when $k_{nc} \leq 0.2\ k_h$ and $k_{nc} \leq 5\ k_h$.

DISCUSSION

The chemical properties of a prodrug can influence its bioavailability and reactivity. The pH and buffer composition of a prodrug solution can affect its hydrolysis rate. Consequently, the UT of a prodrug solution is altered by both the formulation and the bioavailability of the prodrug relative to the drug.

Effect of pH on Prodrug Solution Shelf Life. As observed in Figs. 1 and 2, the UT_{ratio} depends upon the relationship among k_{nc}, k_c, and k_h. Since each of these rate constants would be expected to have a unique pH–rate profile (7), the UT_{ratio} also varies with pH. To examine the UT_{ratio} as a function of pH, it is necessary to have pH-dependent rate expressions for each of the three rate constants. These data were determined for the ampicillin prodrugs, talampicillin and bacampicillin, at 50.5°C, $\mu = 0.5$ (3). Although this does not represent a practical storage condition, these prodrugs illustrate how UT_{ratio} versus pH profiles relate to the two cases discussed following Eq. (6) and the observations in Figs. 1 and 2.

Both talampicillin and bacampicillin are so rapidly and completely converted to ampicillin in the blood (8–12) that intravenous administration would be bioequivalent to ampicillin itself. Figure 3a shows the pH–rate profiles for the talampicillin rate constants, k_c, k_{nc}, and k_{sum}, and the ampicillin hydrolysis constant, k_h. The vertical dashed line shows the pH where $k_h = k_{nc}$.

The data correspond to Case 2 at pH < 3.2, where curve D is less than A, i.e., $k_{nc} < k_h$. The predicted increase in prodrug UT is verified by curve B in Fig. 3b, where the UT_{ratio} exceeds 3.5 at pH < 1.0. At pH values greater than 1.8, k_{sum} (curve B) exceeds k_h. Therefore, the pH region 1.8 to 3.2 represents the case where overall loss of prodrug exceeds that of drug while the prodrug shelf life remains longer than that of the drug as seen by the corresponding pH region in Fig. 3b, where $1.0 < UT_{ratio} < 2.5$. At pH = 3.2, $k_{nc} = k_h$ and therefore the UT_{ratio} is one. Case 1 is illustrated at pH > 3.2, where talampicillin degradation exceeds ampicillin degradation, $k_{nc} > k_h$. Therefore, the storage stability for ampi-

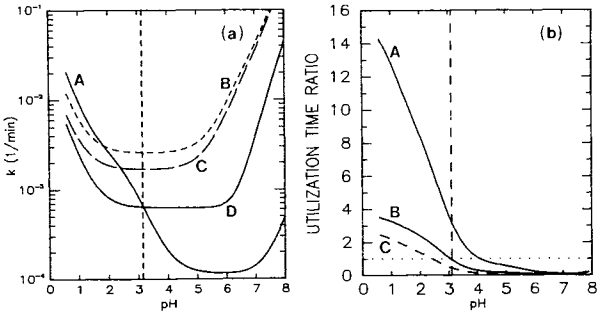

Fig. 3. (a) The pH–rate profiles at 50.5°C, $\mu = 0.5$, for (A) ampicillin degradation, k_h (——); (B) overall loss of talampicillin, k_{sum} (– – –); (C) talampicillin conversion to ampicillin, k_c (— — —); (D) talampicillin degradation, k_{nc} (——). The vertical dashed line divides the pH regions into Cases 1 and 2. (b) The talampicillin UT_{ratio} versus pH profiles at 50.5°C. Curve A is the *oral equimolar* dose, B is the *intravenous* solution, and C is the *oral bioequivalent* dose. The dotted line shows a UT_{ratio} of one. The dashed line shows the pH regions for Cases 1 and 2.

cillin is greater than that for talampicillin ($UT_{ratio} < 1.0$; curve B, Fig. 3b).

A similar treatment was applied to the bacampicillin data in Fig. 4. At pH < 4.5, bacampicillin degradation (curve D) is nearly equal to k_{sum} (curve B) and bacampicillin conversion (curve C) is not significant. Curves A (k_h) and D (k_{nc}) are equal at pH 3.5. At pH < 3.5, k_{nc} is less than k_h and the UT_{ratio} favors bacampicillin (curve B, Fig. 4b). This region illustrates Case 2. At a pH of 3.5, k_{nc} equals k_h, and therefore the UT_{ratio} equals one (curve B, Fig. 4b). At pH > 3.5, k_{nc} is greater than k_h, which corresponds to Case 1, where bacampicillin is less stable than ampicillin and therefore $UT_{ratio} < 1$ (Fig. 4b).

The Influence of Buffer Catalysis. Since the UT_{ratio} is determined by the relative values of k_{nc}, k_c, and k_h, this ratio may be influenced by general acid–base catalysis. Just as each of the three rate constants have unique pH–rate profiles in the absence of general acid–base catalysis (3), the rate constants may be influenced to varying extents by the buffer concentration at a fixed pH. *A priori,* one might expect that the UT_{ratio} could increase, decrease, or remain unchanged in the presence of general acid–base catalysts such as buffer components.

The rate constants, k_c, k_{nc}, and k_h, for bacampicillin and ampicillin in various buffers at 50.5°C, μ = 0.5 (3), were used to calculate the UT_{ratio} values as a function of pH and buffer concentration. The results confirm that buffer catalysis can alter the UT_{ratio}.

At each pH in Fig. 5, the buffer ratio was constant while the total buffer concentration was increased and the pH and ionic strength were maintained constant. At pH 3.52 and 4.64, in acetate buffer, the UT_{ratio} increased with increasing buffer concentration. Because k_h increased to a greater extent than k_{nc}, the T_{90} for ampicillin was reduced to a greater extent by buffer catalysis than was the UT value for bacampicillin. However, at pH 6.56, in phosphate buffer, the UT_{ratio} was depressed by the addition of buffer. At pH 5.64, the acetate buffer also caused a small but observable decrease in the UT_{ratio}. The remaining case, pH 7.52 (phosphate), was insensitive to buffer concentration, probably because prodrug instability resulted in very low UT_{ratio} values, which obscured any changes. Thus, this example illustrates increase, decrease, and independence of the UT_{ratio} on buffer concentration.

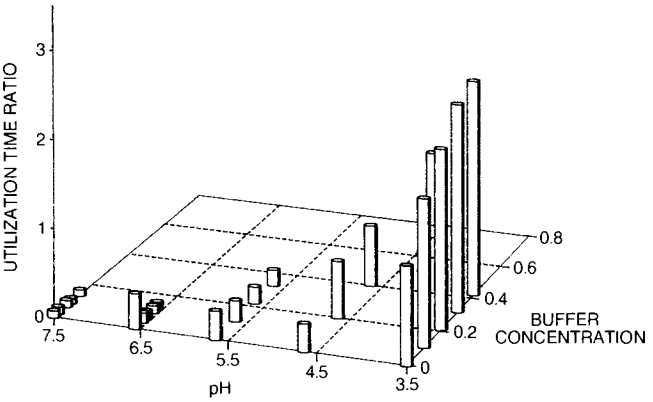

Fig. 5. The UT_{ratio} values for bacampicillin as a function of pH and total buffer concentration at 50.5°C, μ = 0.5. Buffers are acetate at pH 3.52 and 4.64 and phosphate at pH 5.64, 6.56, and 7.52. The observed values for k_c, k_{nc}, and k_h were taken from a previous study (3) to calculate the UT_{ratio} values.

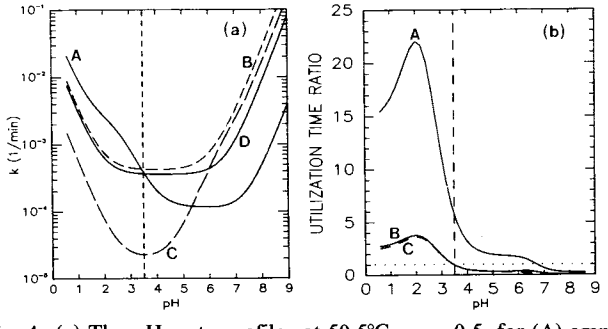

Fig. 4. (a) The pH–rate profiles at 50.5°C, μ = 0.5, for (A) ampicillin degradation, k_h (——); (B) overall loss of bacampicillin, k_{sum} (– – –); (C) bacampicillin conversion to ampicillin, k_c (— — —); (D) bacampicillin degradation, k_{nc} (——). The vertical dashed line divides the pH regions into Cases 1 and 2. (b) The bacampicillin UT_{ratio} versus pH profiles at 50.5°C. Curve A is the *oral equimolar* dose, B is the *intravenous* solution, and C is the *oral bioequivalent* dose. The dotted line shows a UT_{ratio} of one. The dashed line shows the pH regions for Cases 1 and 2.

Bioavailability Considerations. The UT of an extravascular prodrug solution can exceed that of its intravenous solution when the extravascular prodrug dose is absorbed to a greater extent than an equimolar dose of its similarly administered parent drug. This occurs because UT is defined in this report as the time during which the resulting drug plasma concentration–time course equals or exceeds that of the parent drug at its T_{90}. This can be illustrated by considering orally administered solutions of bacampicillin or talampicillin. Oral administration of either bacampicillin or talampicillin provides ampicillin plasma concentration–time courses that show roughly twice the extent of bioavailability with little or no change in curve shape or t_{max} relative to oral ampicillin (8–12).

The minimum acceptable plasma concentration time course is the one achieved by 90% of the bioavailable ampicillin dose, $0.90 f_d D_o$. Consequently, a prodrug solution was considered acceptable for use so long as it produced an ampicillin blood level–time course equal to or greater than that achieved by an acceptable ampicillin solution.

The UT_{ratio} versus pH profiles have been calculated for talampicillin and bacampicillin where the oral dose of a fresh prodrug solution was equimolar to the ampicillin dose. Since both talampicillin, f_{pd} = 0.87 (8,9), and bacampicillin, f_{pd} = 0.89 (10–12), are nearly twice as bioavailable as ampicillin, f_d = 0.50 (1), the improvement in prodrug solution utilization time was significant. Both the talampicillin UT_{ratio} versus pH profile (curve A, Fig. 3b) and the bacampicillin UT_{ratio} versus pH profile (curve A, Fig. 4b) exceeded the intravenous prodrug solutions (B curves) over most of the pH range. The improvement in UT resulted from the fact that a mole of prodrug in an oral solution provided about twice the blood level of a mole of ampicillin. As these prodrugs became unstable relative to ampicillin (pH > 4, curves D in Figs. 3a and 4a), the UT_{ratio} decreased and ultimately fell below one when the improvement in bioavailability failed to compensate for prodrug instability (curves A in Figs. 3b and 4b).

UT_{ratio} versus pH profiles were also generated to represent a prodrug dose which was adjusted to be bioequivalent to the drug dose ($PD'_o = d_d D_o / f_{pd}$). In molar-equivalent oral dosing, the fresh prodrug solutions provided ampicillin plasma concentration–time courses that were superior to those obtained by administration of ampicillin. Since the extent of talampicillin and bacampicillin bioavailability is nearly twice that of ampicillin, the *bioequivalent* prodrug doses were less than the molecular equivalent doses.

Because the oral bioavailability of each molecule of prodrug is equivalent to approximately two molecules of ampicillin, the conversion of prodrug to drug during storage decreases the bioavailable ampicillin. This is different from an intravenous solution, wherein prodrug conversion to ampicillin produced no loss of available ampicillin. For example, during 10% prodrug conversion to drug without loss to degradation, the oral bioactivity from 10% prodrug is replaced by ampicillin with a net loss of 5%.

Therefore, the UT_{ratio} versus pH profile for talampicillin was significantly reduced (curve C, Fig. 3b) using a bioequivalent dose. As seen in Fig. 3a, talampicillin was lost primarily through conversion over the entire pH range. While conversion maintained potency for the intravenous solution so long as $k_{nc} < k_h$, an oral solution loses potency via conversion resulting in a decreased UT_{ratio} (compare curves B and C in Fig. 3b). Conversely, when $k_{nc} < k_h$ for bacampicillin (Fig. 4a, pH < 3.5), overall loss was due primarily to degradation (k_{nc}). Therefore, there was no difference between the UT_{ratio} of the intravenous and the oral solution in this region (compare curve B to curve C in Fig. 4b).

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