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Degradation kinetics of the investigational antitumor compound LL-D49194α_1 in the presence of buffers

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Abstract

The degradation kinetics of LL-D49194α_1 were studied over the pH region 1–13. The contribution of the buffering agents to the degradation has been analyzed using appropriate model equations.

Keywords: Degradation kinetics; Buffer-catalyzed degradation; General acid-base catalysis; LL-D49194α_1

LL-D49194α_1 (LLD, Fig. 1) is an antibiotic which has been shown to have antibacterial and antitumor activity (Lee et al., 1986). Although cardiotoxicity has been observed recently (Cassidy et al., 1993) the substance is still under further investigation. It has been reported to be unstable in solution (Lee et al., 1986), however, no details of the degradation process are available in the literature. Preliminary investigations gave indications for buffer-catalyzed degradation.

LLD (Lederle, American Cyanamid Laboratories, Pearl River, U.S.A.) was used as such. All other chemicals were of analytical grade. For the kinetic studies the following aqueous buffer solutions were used: pH 1–3, perchloric acid; pH 3–6, acetate; pH 6–9, phosphate; pH 9–11, carbonate; pH > 11, sodium hydroxide. A constant ionic strength of 0.3 was maintained for each solution by addition of an appropriate amount of sodium chloride, except for solutions where the hydroxyl concentration exceeded 0.3 M. The kinetic experiments were performed at 25 ± 0.2°C, protected from light. The reactions were initiated by adding 50 μl of a methanolic stock solution (2 mg/ml) to 2 ml pre-heated buffer solution. At appropriate time intervals 20 μl samples were taken and analyzed directly by HPLC using a stainless-steel column (12.5 × 4.0 mm i.d.), containing Lichrosorb RP8 (5 μm) material (Merck, Darmstadt, Germany) and a mixture of 5×10^{-3} M potassium phosphate (pH 7) and methanol (45 : 55, v/v) as the eluent. Detection was carried out at 254 and 405 nm.

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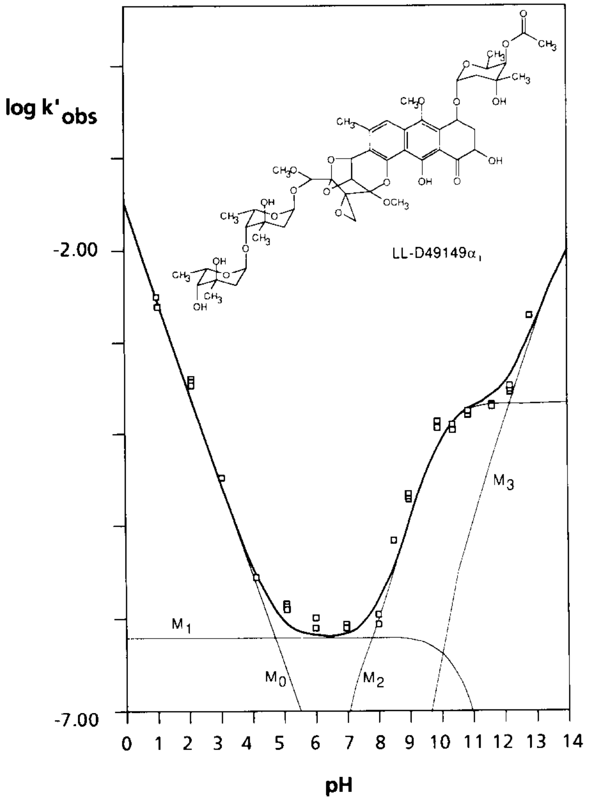

Fig. 1. Model and contribution of the macro-reaction constants to the overall degradation profile of LDD in the absence of buffers.

For the determination of the pK_a value of LLD the absorbance of a solution of 33.3 μg/ml was measured at different pH values at 270 nm. Using Eq. 1 as model equation a pK_a value of 10.3 ± 0.1 was calculated.

$$A = C \cdot \frac{\epsilon_0 + \epsilon_1 \cdot \dfrac{K_a}{[H^+]}}{1 + \dfrac{K_a}{[H^+]}} \tag{1}$$

The sum of the molar concentrations of the protonated and deprotonated LLD is given by C. For the molar extinction coefficients of these species (indicated by ϵ_0 and ϵ_1) values of 3.90×10^4 and 2.42×10^4 were obtained.

The degradation rate constant, k_{obs}, was measured at constant ionic strength for a range of buffer concentrations.

The degradation profile is described by Eq. 2 if the solute is subject to a single and the buffer is subject to three and by Eq. 3 if the buffering agent is involved in two successive protolytic equilibria (Van der Houwen et al., 1988, 1994).

$$k_{obs} = \frac{M_0 \cdot [H^+] + M_1 + \dfrac{M_2}{[H^+]} + \dfrac{M_3}{[H^+]^2}}{1 + \dfrac{K_a}{[H^+]}} + C_{buf} \cdot \frac{M_0^B + \dfrac{M_1^B}{[H^+]} + \dfrac{M_2^B}{[H^+]^2} + \dfrac{M_3^B}{[H^+]^3} + \dfrac{M_4^B}{[H^+]^4}}{1 + \dfrac{K_1^c}{[H^+]} + \dfrac{K_2^c}{[H^+]^2} + \dfrac{K_3^c}{[H^+]^3} + \dfrac{K_4^c}{[H^+]^4}} \tag{2}$$

The observed rate constant is a linear function of the buffer concentration C_{buf}. The first part of Eq. 2 and 3:

$$k_{obs} = \frac{M_0 \cdot [H^+] + M_1 + \dfrac{M_2}{[H^+]} + \dfrac{M_3}{[H^+]^2}}{1 + \dfrac{K_a}{[H^+]}} + C_{buf} \cdot \frac{M_0^B + \dfrac{M_1^B}{[H^+]} + \dfrac{M_2^B}{[H^+]^2} + \dfrac{M_3^B}{[H^+]^3}}{1 + \dfrac{K_1^c}{[H^+]} + \dfrac{K_2^c}{[H^+]^2} + \dfrac{K_3^c}{[H^+]^3}} \tag{3}$$

(the intercept) corresponds with the buffer-independent, and the second part of these equations (the slope) with the buffer-dependent contribution to the degradation. When the buffer concentration is varied at fixed pH and fixed ionic strength the contribution of both parts can be calculated.

The buffer-independent contribution was analyzed using Eq. 4 as a model:

$$k_{obs} = \frac{M_0 \cdot [H^+] + M_1 + \dfrac{M_2}{[H^+]} + \dfrac{M_3}{[H^+]^2}}{1 + \dfrac{K_a}{[H^+]}} \tag{4}$$

Table 1
Macro-reaction constants and K_a value

pK_a value of LLD	
K_a	$5.4 \pm 2.2 \times 10^{-11}$ M^{-1} (pK_a 10.3 ± 0.2)
Macro-reaction constants of the buffer-independent degradation of LLD	
M_0	$3.2 \pm 0.5 \times 10^{-2}$ M^{-1} s^{-1}
M_1	$6.4 \pm 1.4 \times 10^{-7}$ s^{-1}
M_2	$9.1 \pm 2.3 \times 10^{-15}$ M^{1} s^{-1}
M_3	$5.4 \pm 3.5 \times 10^{-27}$ M^{2} s^{-1}
Macro-reaction constants of the buffer-catalyzed degradation	
Phosphate-catalyzed degradation	
M_0^B	$5.4 \pm 0.8 \times 10^{-3}$ s^{-1}
M_1^B	$3.8 \pm 2.5 \times 10^{-9}$ M^{1} s^{-1}
M_2^B	$9.0 \pm 0.7 \times 10^{-15}$ M^{2} s^{-1}
M_3^B	–
M_4^B	–
Carbonate-catalyzed degradation	
M_0^B	–
M_1^B	$9.1 \pm 15.5 \times 10^{-12}$ M^{2} s^{-1}
M_2^B	$6.8 \pm 0.5 \times 10^{-20}$ M^{2} s^{-1}
M_3^B	–

The macro-reaction constants $M_0 - M_3$ and the K_a value, are given in Table 1. Their contribution to the overall degradation profile is illustrated in Fig. 1. The degradation profile is almost entirely described by the first three macro-reaction constants ($M_0 - M_2$). The fourth macro-reaction (M_3) only contributes to the pH profile above pH 12. The pK_a value calculated from the degradation profile (10.3 ± 0.2) corresponds well with that from the pH-absorbance profile (10.3 ± 0.1).

The macro-reaction constants are related to the individual reaction constants according to Eq. 5–8:

$$M_0 = k^{H}_{HLLD} \tag{5}$$

$$M_1 = k^{H}_{LLD^-} \cdot K_a + k^{S}_{HLLD} \tag{6}$$

$$M_2 = k^{S}_{LLD^-} \cdot K_a + k^{OH}_{HLLD} \cdot K_w \tag{7}$$

$$M_3 = k^{OH}_{LLD^-} \cdot K_a \cdot K_W \tag{8}$$

The superscripts H, S and OH refer to the proton-, solvent- and hydroxyl-catalyzed reactions, and the subscripts HLLD and LLD$^-$ denote the protonated and deprotonated species of LLD, respectively. The macro-reaction constants M_1 and M_2 are both related to two kinetically indistinguishable micro-reactions. The contribution of the separate reactions therefore cannot be calculated from the macro-reaction constant. However, it is possible to estimate the hypothetical maximum possible contribution of each reaction neglecting the contribution of the other reaction. In this way, an indication can be obtained of the order of magnitude of the micro-reaction constant necessary to contribute significantly to the observed degradation. The calculated values thus obtained are given in Table 2. The calculated limiting value of $k^{H}_{LLD^-}$ is, compared to the other micro-reaction constants, extremely high, which suggests that it is improbable for this micro-reaction to contribute significantly to the macro-reaction. Its high value reflects the low value of the product $[H^+] \cdot [LLD^-]$ due to the high pK_a.

The contributions of the buffer to the degradation were calculated as the slope of the plot of k_{obs} vs C (Eq. 2 and 3). The obtained values were

Table 2
Reaction constants and micro-reaction constants

Calculated values of the reaction constants of the proton-, solvent- and hydroxyl-catalyzed degradation reaction of LLD	
K^{H}_{HLLD}	3.2×10^{-2} M^{-1} s^{-1}
K^{S}_{HLLD}	6.4×10^{-7} s^{-1} [a]
K^{OH}_{HLLD}	9.1×10^{-1} M^{-1} s^{-1} [a]
$K^{H}_{LLD^-}$	1.4×10^{-4} M^{-1} s^{-1} [a]
$K^{S}_{LLD^-}$	2.0×10^{-4} s^{-1} [a]
$K^{OH}_{LLD^-}$	1.2×10^{-2} M^{-1} s^{-1}
Individual micro-reaction constants contributing to the buffer-catalyzed degradation of LLD	
$K^{H_3PO_4}_{HLLD}$	5.4×10^{-3} M^{-1} s^{-1}
$K^{H_2PO_4^-}_{HLLD}$	$< 8 \times 10^{-1}$ M^{-1} s^{-1} [a]
$K^{HPO_4^{2-}}_{HLLD}$	2.0×10^{-5} M^{-1} s^{-1} [a]
$K^{H_3PO_4}_{LLD}$	$< 5 \times 10^{-7}$ M^{-1} s^{-1} [a]
$K^{H_2PO_4^-}_{LLD^-}$	2.6×10^{-2} M^{-1} s^{-1} [a]
$K^{HCO_3^-}_{HLLD}$	$< 2 \times 10^{-1}$ M^{-1} s^{-1} [a]
$K^{CO_3^{2-}}_{HLLD}$	2.4×10^{-3} M^{-1} s^{-1} [a]
$K^{H_2CO_3}_{LLD}$	$< 2 \times 10^{-5}$ M^{-1} s^{-1} [a]
$K^{HCO_3^-}_{LLD^-}$	3.4×10^{-3} M$_{-1}$ s^{-1} [a]

[a] Values obtained by neglecting the corresponding kinetically indistinguishable reaction.

subsequently analyzed using Eq. 9 and 10 as model equations for phosphate and carbonate buffer, respectively.

$$\text{slope} = \frac{M_0^B + \dfrac{M_1^B}{[H^+]} + \dfrac{M_2^B}{[H^+]^2} + \dfrac{M_3^B}{[H^+]^3} + \dfrac{M_4^B}{[H^+]^4}}{1 + \dfrac{K_1^c}{[H^+]} + \dfrac{K_2^c}{[H^+]^2} + \dfrac{K_3^c}{[H^+]^3} + \dfrac{K_4^c}{[H^+]^4}} \tag{9}$$

$$\text{slope} = \frac{M_0^B + \dfrac{M_1^B}{[H^+]} + \dfrac{M_2^B}{[H^+]^2} + \dfrac{M_3^B}{[H^+]^3}}{1 + \dfrac{K_1^c}{[H^+]} + \dfrac{K_2^c}{[H^+]^2} + \dfrac{K_3^c}{[H^+]^3}} \tag{10}$$

The pK_a values for the phosphate and the carbonate buffers were taken from the literature (Weast, 1988). For the phosphate model the constants K_i^c were calculated with Eq. 11–14, and for the carbonate model with Eq. 15–17 (Van der Houwen et al., 1994):

$$K_1^c = K_a^{LLD} + K_{a1}^{phosphate} \tag{11}$$

$$K_2^c = K_a^{LLD} \cdot K_{a1}^{phosphate} + K_{a1}^{phosphate} \cdot K_{a2}^{phosphate} \tag{12}$$

$$K_3^c = K_a^{LLD} \cdot K_{a1}^{phosphate} \cdot K_{a2}^{phosphate} + K_{a1}^{phosphate} \cdot K_{a2}^{phosphate} \cdot K_{a3}^{phosphate} \tag{13}$$

$$K_4^c = K_a^{LLD} \cdot K_{a1}^{phosphate} \cdot K_{a2}^{phosphate} \cdot K_{a3}^{phosphate} \tag{14}$$

$$K_1^c = K_a^{LLD} + K_{a1}^{carbonate} \tag{15}$$

$$K_2^c = K_a^{LLD} \cdot K_{a1}^{carbonate} + K_{a1}^{carbonate} \cdot K_{a2}^{carbonate} \tag{16}$$

$$K_3^c = K_a^{LLD} \cdot K_{a1}^{carbonate} \cdot K_{a2}^{carbonate} \tag{17}$$

The calculated macro-reaction constants M_0^B–M_4^B are given in Table 1. For the phosphate buffer the macro-reaction constants M_0^B and M_2^B contribute significantly to the degradation profile. The contribution of M_1^B is hardly significant as can be seen from the estimated error. Its calculated value is therefore not more than an indication of the upper possible limit of its order of magnitude.

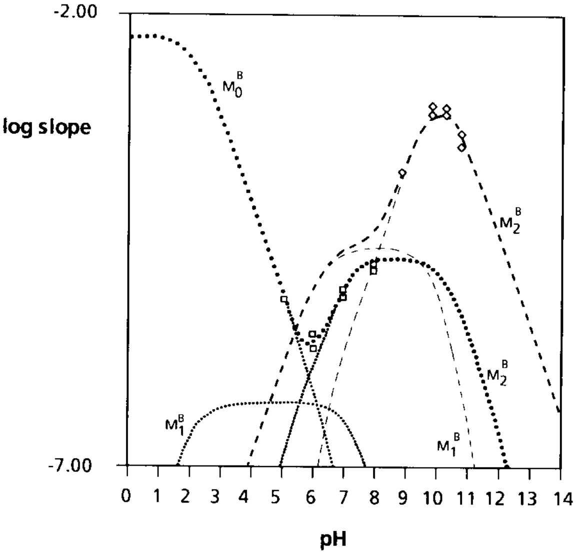

Fig. 2. Model and contribution of the macro-reaction constants to the phosphate-catalyzed (dotted line), and the cabonate-catalyzed (dashed line) degradation.

No reasonable estimates of M_3^B and M_4^B can be made in the pH range studied. This is a problem inherent to the fact that buffers can only be used in the limited range of pH = pK_a ± 2 units.

For the carbonate buffer only M_2^B contributes significantly to the degradation. The contribution of the constant M_1^B is at best an estimation of the upper limit of its order of magnitude. The contributions of the macro-reaction constants to the buffer-dependent degradation are illustrated in Fig. 2.

The relation of the reaction constants of the individual reactions and the macro-reaction constants is given by Eq. 18–22 for the phosphate buffer.

$$M_0^B = k_{HLLD}^{H_3PO_4} \tag{18}$$

$$M_1^B = k_{HLLD}^{H_2PO_4^-} \cdot K_a^{LLD} + k_{HLLD^-}^{H_3PO_4} \cdot K_{a1}^{phosphate} \tag{19}$$

$$M_2^B = k_{HLLD}^{HPO_4^{2-}} \cdot K_{a1}^{phosphate} \cdot K_{a2}^{phosphate} + k_{LLD^-}^{H_2PO_4^-} \cdot K_a^{LLD} + K_{a1}^{phosphate} \tag{20}$$

$$M_3^{\mathrm{B}} = k_{\mathrm{HLLD}}^{\mathrm{PO_4^{3-}}} \cdot K_{\mathrm{a1}}^{\mathrm{phosphate}} \cdot K_{\mathrm{a2}}^{\mathrm{phosphate}} \cdot K_{\mathrm{a3}}^{\mathrm{phosphate}} + k_{\mathrm{LLD^-}}^{\mathrm{HPO_4^{2-}}} \cdot K_{\mathrm{a}}^{\mathrm{LLD}} + K_{\mathrm{a1}}^{\mathrm{phosphate}} \cdot K_{\mathrm{a2}}^{\mathrm{phosphate}} \tag{21}$$

$$M_4^{\mathrm{B}} = k_{\mathrm{LLD^-}}^{\mathrm{PO_4^{3-}}} \cdot K_{\mathrm{a}}^{\mathrm{LLD}} \cdot K_{\mathrm{a1}}^{\mathrm{phosphate}} \cdot K_{\mathrm{a2}}^{\mathrm{phosphate}} \cdot K_{\mathrm{a3}}^{\mathrm{phosphate}} \tag{22}$$

and by Eq. 23–26 for the carbonate buffer:

$$M_0^{\mathrm{B}} = k_{\mathrm{HLLD}}^{\mathrm{H_2CO_3}} \tag{23}$$

$$M_1^{\mathrm{B}} = k_{\mathrm{HLLD}}^{\mathrm{HCO_3^-}} \cdot K_{\mathrm{a}}^{\mathrm{LLD}} + k_{\mathrm{LLD^-}}^{\mathrm{H_2CO_3}} \cdot K_{\mathrm{a1}}^{\mathrm{carbonate}} \tag{24}$$

$$M_2^{\mathrm{B}} = k_{\mathrm{HLLD}}^{\mathrm{CO_3^{2-}}} \cdot K_{\mathrm{a1}}^{\mathrm{carbonate}} \cdot K_{\mathrm{a2}}^{\mathrm{carbonate}} + k_{\mathrm{LLD^-}}^{\mathrm{HCO_3^-}} \cdot K_{\mathrm{a}}^{\mathrm{LLD}} + K_{\mathrm{a1}}^{\mathrm{carbonate}} \tag{25}$$

$$M_3^{\mathrm{B}} = k_{\mathrm{LLD^-}}^{\mathrm{CO_3^{2-}}} \cdot K_{\mathrm{a}}^{\mathrm{LLD}} \cdot K_{\mathrm{a1}}^{\mathrm{carbonate}} \cdot K_{\mathrm{a2}}^{\mathrm{carbonate}} \tag{26}$$

The species of LLD involved in the reaction are indicated by the subscripts of the reaction constant, the catalyzing buffer species being indicated by the superscript of the reaction constant.

The calculated reaction constants of the individual reactions are listed in Table 2. In the case where the macro-reaction constant was related to two kinetically indistinguishable reactions the maximal possible contribution of each reaction (neglecting the contribution of the corresponding reaction) was calculated.

The results obtained from the calculations indicate that it has been possible to analyze the pH degradation profile of LLD in both the absence and presence of degradation-catalyzing buffer species. The pH profile in the absence of buffer could be explained over the pH range of 0–14.

The buffer-dependent degradation could, according to the nature of the use of buffers, only be investigated over short pH ranges. The linear relationship between buffer concentration and its contribution to the degradation rate enables to separate the contribution of buffer-catalyzed degradation from proton-, solvent- and hydroxyl-catalyzed degradation.

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