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## Computation of mitomycin C- $\gamma$ -cyclodextrin complex stability constant

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In a preliminary paper from our laboratory we reported the stabilization of mitomycin C (MMC) (Fig. 1) on complexation with  $\gamma$ -cyclodextrin ( $\gamma$ -CyD) in aqueous acidic solutions (Bekers et al., 1989). Apart from effects on the stability of MMC, complex stability constants,  $K_s$ , were also obtained by using the Lineweaver-Burk equation (Lineweaver and Burk, 1934). These  $K_s$  values, however, appeared to be dependent on the pH of the degradation media (Table 1), which is inconsistent with the nature of this equilibrium constant. The reason for this influence is the fact that at pH values around the  $pK_a$  of MMC ( $pK_a = 2.8$ ; Beijnen et al., 1986) two species are present: a cationic species ( $MMCH^+$ ) and a neutral species (MMC). Probably only the latter will be subject to  $\gamma$ -CyD complexation. Moreover, the rate constants for degradation of both species are substantially different, which turns  $k_{obs}^0$ , the observed rate constant for the free drug, into a heterogeneous, pH-dependent term. For this reason, the Lineweaver-Burk equation cannot be employed straightforwardly to obtain reliable values for  $K_s$ . Values for  $K_s$  found in the pH region  $0.8 < \text{pH} < 4.8$  are only constant at constant pH

and are, therefore, referred to as apparent complex stability constants,  $K'_s$ . At pH values  $> 4.8$ , the  $[\text{MMH}^+]$  becomes negligible and, hence, it can be expected that values for  $K'_s$  in this pH region approach the value of the real constant  $K_s$ .

To overcome these problems a theoretical approach has recently been developed (Van der Houwen et al., 1991), to calculate complex stability constants for pH-dependent degradations of polybasic weak electrolytes in the presence of a ligand. In the present study, this approach has been applied to MMC complexation with  $\gamma$ -CyD in acidic solutions.

The experimental conditions are described here only briefly; a detailed description has been published in our earlier report (Bekers et al., 1989). On degradation in acidic media MMC shows profound spectral changes. These changes, among which a decrease in absorbance at 363 nm, are suitable to monitor the acidic MMC degradation and to calculate degradation constants. The presence  $\gamma$ -CyD does not alter this spectral behaviour. All kinetic experiments were performed in the dark at 25°C. In the  $H_0/\text{pH}$  range 0.3–3.0 perchloric acid solutions and in the pH range 3.0–4.8 acetate buffers (0.001 M) were used.

The degradations were followed at fixed  $H_0/\text{pH}$  values varying from 0.3 to 4.8, at every

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pH with six different  $\gamma$ -CyD concentrations between 0 and  $5 \times 10^{-2}$  M.

The dependence of the observed rate constant,  $k_{\text{obs}}$ , on the  $\gamma$ -CyD concentration is given in Eqn 1 (Van der Houwen et al., 1991)

$$k_{\text{obs}} = \left[ k_{\text{obs}}^{\text{o}} + [\text{CyD}] \cdot \sum_{i=0}^{n+2} (M_i^{\text{CyD}} / [\text{H}^+])^{i-1} \right]$$

$$\left/ \sum_{i=0}^n \left\{ \left( \bigcap_{j=0}^i K_j \right) / [\text{H}^+]^i \right\} \right]$$

$$\left/ \left[ 1 + [\text{CyD}] \cdot \sum_{i=0}^n \left\{ \left( \bigcap_{j=0}^i K_j \right) \cdot K_i^{\text{CyD}} / [\text{H}^+]^i \right\} \right] \right.$$

$$\left/ \sum_{i=0}^n \left\{ \left( \bigcap_{j=0}^i K_j \right) / [\text{H}^+]^i \right\} \right] \quad (1)$$

where  $M_i^{\text{CyD}}$  represents the contribution of the degradation of the CyD bound species to the  $i$ -th macroreaction constant  $M_i$ .

$$\bigcap_{j=0}^i K_j = \text{product } K_0 \cdot K_1 \cdot K_2 \cdots K_i$$

and  $K_i^{\text{CyD}}$  denotes the complex stability constant of the  $i$ -th species of the solute.

This equation can be used for non-linear regression analysis for a series of measurements of the  $k_{obs}$  at varying CyD concentrations at fixed pH.

TABLE I

### MMC- $\gamma$ -CyD apparent complex stability constants at various pH values

pH	$K_s' (M^{-1})$
0.3	a
0.8	a
1.8	48
2.8	107
3.8	215
4.8	249

<sup>a</sup>  $K_c$  not determined since no stabilization occurs

Eqn 1 can be written in a simplified form:

$$k_{\text{obs}} = \frac{k_{\text{obs}}^0 + A \cdot [\text{CyD}]}{1 + B \cdot [\text{CyD}]} \quad (2)$$

The values of  $A$  and  $B$ , calculated from series of measurements at a number of fixed pH values, are themselves functions of the pH. The contributing constants  $M_i^{\text{CyD}}$  and  $K_i^{\text{CyD}}$  can be extracted from the values of  $A$  and  $B$  obtained, using the functions in the numerator and denominator, respectively, of Eqn 1 by linear regression.

The values of  $A$  and  $B$  were calculated (Table 2) and used in Eqn 1 to compute the MMC- $\gamma$ -CyD complex stability constant. By using a model for one protolytic equilibrium, as in this case, a MMC- $\gamma$ -CyD complex stability constant of 316  $M^{-1}$  has been calculated. This value is, as had already been expected, in reasonable agreement with the  $K_s'$  obtained for MMC at pH 4.8, where 1% of the total concentration of MMC still exists in the protonated state. This indicates that the

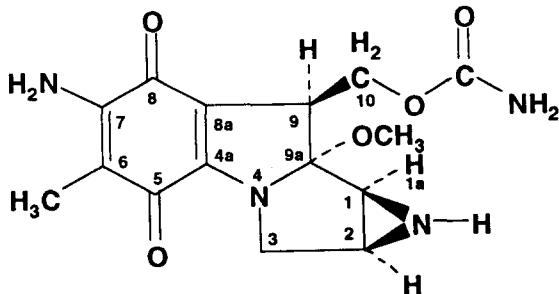


Fig. 1. Structure of mitomycin C.

TABLE 2

Calculated A and B coefficients of MMC in the presence of  $\gamma$ -CyD at various pH values

pH	<i>A</i>	<i>B</i>
0.3	$1.06 \times 10^{-1}$	$3.28 \times 10^1$
0.8	$5.23 \times 10^{-1}$	$1.27 \times 10^1$
1.8	$3.27 \times 10^{-2}$	$3.86 \times 10^1$
2.8	$1.16 \times 10^{-2}$	$3.93 \times 10^2$
3.3	$3.51 \times 10^{-3}$	$2.55 \times 10^2$
4.8	$1.58 \times 10^{-3}$	$3.93 \times 10^2$

theoretical approach developed can be used for practical situations. To prove this extensively, more studies, with various polybasic weak electrolytes in the presence of different CyDs, are currently in progress.

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