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STABILITY OF [(*N*-MORPHOLINE)METYLENE]DAUNORUBICIN HYDROCHLORIDE IN SOLID STATE

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Abstract: The influence of temperature and relative air humidity on the stability of the novel derivative of daunorubicin: [(*N*-morpholine)metylene]daunorubicin hydrochloride was investigated. The process of degradation was studied by using high-performance liquid chromatography with ultraviolet (UV) detection. In dry air, the degradation of [(*N*-morpholine)metylene]daunorubicin hydrochloride was a first-order reaction depending on the substrate concentration, while at relative air humidity from 60.5 to 90.0% it was an autocatalytic reaction of the first order with respect to MMD concentration. The kinetic and thermodynamic parameters of degradation were calculated.

Keywords: [(*N*-morpholine)metylene]daunorubicin hydrochloride, stability in solid state, kinetic and thermodynamic parameters

Antracyclines are very effective anticancer drugs, but their clinical use is limited by their toxic effect on healthy tissue and the drug resistance of tumor cells (1). Chemical modification of a molecule can be one of the ways to reduce its cardiotoxicity and to overcome the resistance of cancer cells to the drug (2). [(*N*-morpholine)metylene]daunorubicin hydrochloride (MMD) (Fig. 1) was synthesized by the replacement of the primary amino group at C-3' of the daunosamine moiety in daunorubicin by an amidine substituent.

During *in vitro* and *in vivo* studies MMD has demonstrated cytotoxic effect comparable to that of daunorubicin and less cardiotoxicity than daunorubicin (3, 4). An isocratic HPLC method with UV detection for determination of four derivatives of daunorubicin has been developed and validated (5). Previous studies have proved that daunorubicin and its new derivatives are susceptible to degradation in solid state (6) and in aqueous solution (7-9). This publication is concerned with the stability of MMD in its solid state as a function of temperature and humidity (RH) if compared to daunorubicin itself.

EXPERIMENTAL

Materials and methods

MMD was obtained from the Institute of Biotechnology and Antibiotics in Warszawa. All chemicals (Sigma-Aldrich) and all solvents (POCH) were either reagents or HPLC grade. High quality pure water was supplied by using the Millipore purification system (Millipore, Molsheim, France, model Exil SA 67120).

Chromatographic separation and quantitative analysis were performed by using an HPLC method. The analytical system consisted of a quaternary pump (L-7100), an autosampler (L-7200), a column oven (L-7360) and diode array detector (L-7455) (all Merck Hitachi products). A LiChrospher RP 18e column (125 mm × 4 mm, particle size 5 μm, Merck, Darmstad, Germany) was used as the stationary phase. The mobile phase consisted of a mixture of 9 volumes of acetonitrile, 1 volume of methanol and 10 volumes of a solution containing 2.88 g/L of sodium lauryl sulfate and 1.6 mL/L of phosphoric(V) acid. The flow rate was 2.0 mL/min. The detection wave-

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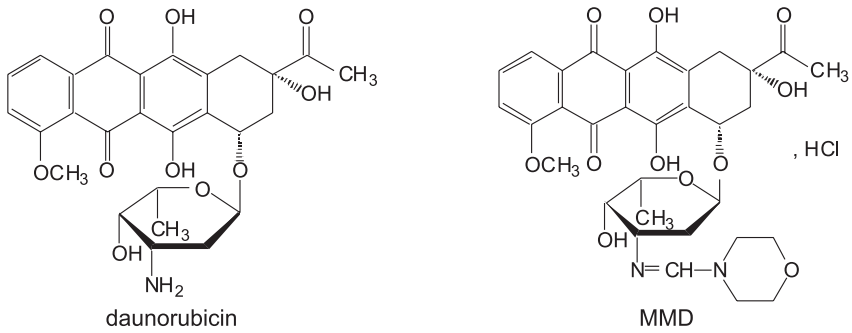
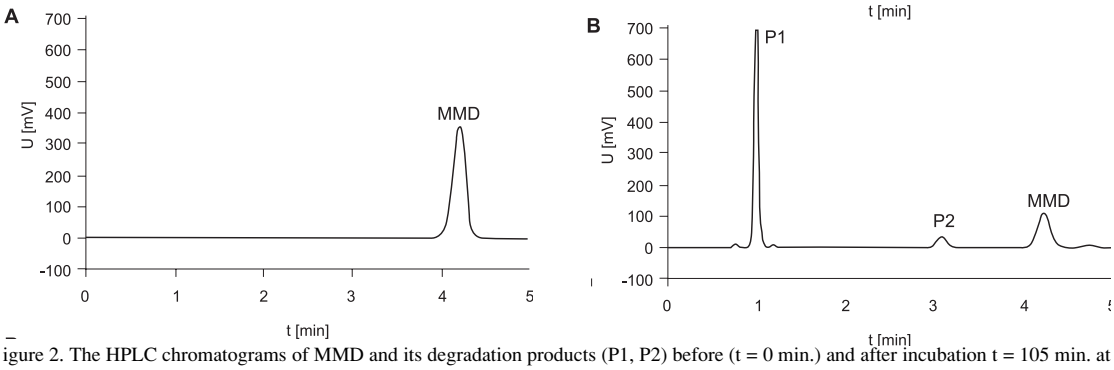


Figure 1. The chemical structure of daunorubicin and [(*N*-morpholine)metylene]daunorubicin hydrochloride (MMD)



igure 2. The HPLC chromatograms of MMD and its degradation products (P1, P2) before (t = 0 min.) and after incubation t = 105 min. at RH = 90% and at 353 K

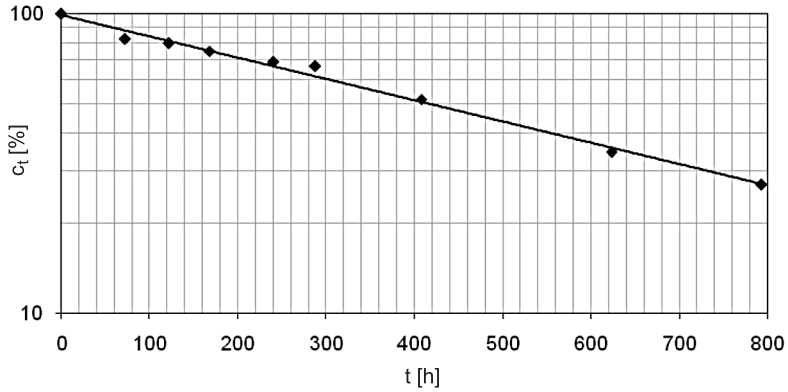


Figure 3. Semilogarithmic plot $c_t = f(t)$ for the degradation of MMD at RH = 0% at 373 K

length was 254 nm. Chromatographic separations were performed at 30°C. Although the used method has been evaluated and validated for determination of four derivatives of daunorubicin in aqueous solutions (5), the selectivity was examined for intact and degraded samples at elevated temperatures (Fig. 2).

Kinetic procedure

Stability tests were performed according to International Conference on Harmonization Guidelines (10).

Five mg samples of MMD have been weighted into 5 mL vials. The samples of examined substances tested for the influence of temperature in a humid environment were placed in incubators set to the desired temperatures (343, 353, 363 and 373 K) and humidity (60.5, 66.5, 76.4 and 90.0%).

To evaluate the stability of MMD in the dry air, the vials containing 5.0 mg of this substance were immersed in a sand bath placed in heat chambers at 373 K.

Each bath to be studied comprised 8–12 samples. At specific time intervals, determined by the

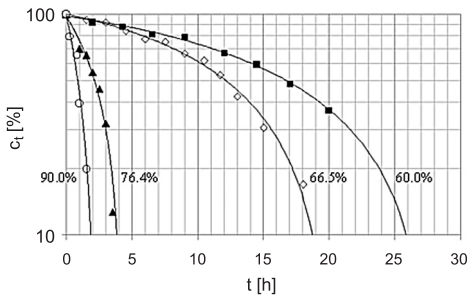


Figure 4. Semilogarithmic plot $c_t = f(t)$ for the degradation of MMD at different RH at 363 K

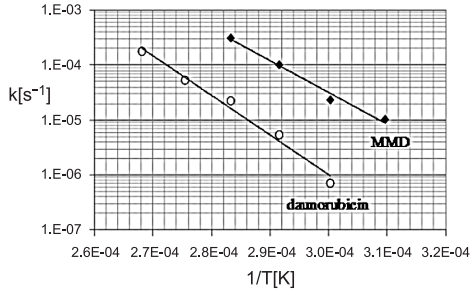


Figure 6. Semilogarithmic relationship $k = f(1/T)$ for the degradation of MMD and daunorubicin at 76.4% RH

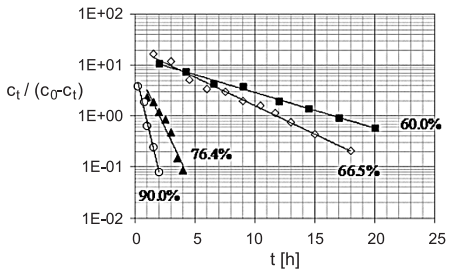


Figure 5. Semilogarithmic plot $c_t/(c_0 - c_t) = f(t)$ for the degradation of MMD at different RH at 363 K.

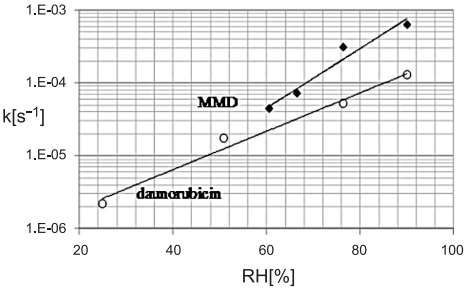


Figure 7. Semilogarithmic relationship $\ln k = f(RH\%)$ for MMD at 353 K and daunorubicin at 363 K both in the solid state

rate of degradation, the vials were removed, cooled to room temperature and the contents dissolved in a mixture (1:1) of acetonitrile and water. The so obtained solutions were quantitatively transferred into volumetric flasks and completed to a total volume of 10.0 mL with the same mixture of solvents. After filtration, 25 µL samples were injected onto the column.

RESULTS AND DISCUSSION

Changes in the concentration of MMD under the conditions of the study were evaluated using the HPLC method. This method was found selective for the determination of MMD in presence of its degradation products (Fig. 2). In the chromatograms of MMD developed over a period of 0 to 5 min, the following peaks emerged: peak MMD, with retention time of approx. 4.12 min; and peaks of the degradation products, with retention time of approx. 0.97 min and 2.93 min.

Kinetics of MMD degradation

The degradation of MMD in dry air (RH = 0%) was a first-order reaction depending on the substrate concentration (Fig. 3) and the rate constants were calculated from the following equation:

$$\ln c_t = \ln c_0 - k_{obs} \times t \quad (1)$$

where c_t and c_0 are the concentrations of MMD, at time t = 0 and t, respectively, and k_{obs} is the rate constant of degradation reaction.

The rate constant of MMD degradation at 373 K and 0% RH was $(4.55 \pm 0.39) \times 10^{-7}$ s^{-1}.

The degradation of MMD at an increased temperature and relative air humidity (RH > 50%) was an autocatalytic reaction of the first order with respect to MMD concentration (Fig. 4)

The changes in the concentration of MMD were not linear because in this reaction model an induction phase with a very small substrate loss is initially observed, which is followed by an acceleration period that involves a rapid substrate degradation.

The semilogarithmic plots $c_t/(c_0 - c_t) = f(t)$ (Fig. 5) were straight-lines and their slopes corresponded to the rate constants of the reaction ($-k_{obs}$) which were calculated from the equation:

$$\ln c_t/(c_0 - c_t) = -k_{obs} \times t + C \quad (2)$$

where c_0 and c_t are concentrations of MMD at times t_0 and t; $c_0 - c_t$ are products concentrations at time t;

Table 1. Kinetic and thermodynamic parameters of the degradation of MMD and daunorubicin (6) in solid state at 76.4% RH.

T [K]	10^6 (k ± Δk) [s^{-1}]	Statistical evaluation lnk = f(1/T)	Thermodynamic parameters
MMD			
323	(10.3 ± 2.3)	a = –13316 ± 4639	E_a = 110 ± 39 (kJ/mol)
333	(23.7 ± 2.5)	S_a = 1078	$\Delta H^{\neq a}$ = 108 ± 41 (kJ/mol)
343	(101 ± 7)	b = 29.6 ± 13.7	$\Delta S^{\neq a}$ = 1 ± 130 (JK^{-1} mol^{-1})
353	(316 ± 87)	S_b = 3.19	
		r = 0.9935 S_y = 0.21	
DAUNORUBICIN (6)			
333	(0.712 ± 0.081)	a = –16581 ± 3972	E_a = 138 ± 33 (kJ/mol)
343	(5.32 ± 0.71)	S_a = 1248	$\Delta H^{\neq a}$ = 135 ± 35 (kJ/mol)
353	(22.6 ± 3.8)	b = 35.94 ± 11.3	$\Delta S^{\neq a}$ = –149 ± 203 (JK^{-1} mol^{-1})
363	(52.5 ± 19.5)	S_b = 3.5	
373	(175 ± 2)	r = 0.9916 S_y = 0.3178	

E_a – activation energy; $\Delta H^{\neq}$ – enthalpy; $\Delta S^{\neq}$ – entropy; $Ea = -aR$; $\Delta H^{\neq} = E_a - TR$; $\Delta S^{\neq} = R(lnA\ ln(k_bT)/h$ where: k_B – Boltzmann's constant (1.3807 10^{-23} JK^{-1}); h – Planck's constant (6.626 10^{-34} Js); R – universal gas constant (8.314 $K^{-1}mol^{-1}$), T – temperature [K]; a –, vectorial coefficient of the Arrhenius relationship; A – frequency coefficient. [a] calculated for 298 K.

Table 2. The effect of relative air humidity on the stability of MMD and daunorubicin (6) at 363 K.

Relative air humidity (%)	10^5 (k ± Δk) [s^{-1}]	Statistical evaluation lnk = f(RH%)
MMD		
60.5	(4.47 ± 0.32)	$a = (9.42 \pm 6.37) \times 10^{-2}$
66.5	(7.15 ± 0.59)	$S_a = 1.48 \times 10^{-2}$
76.4	(31.6 ± 8.7)	b = -15.65 ± 4.73 S_b = 1.099
90.0	(63.7 ± 14.6)	r = 0.9762 S_y = 0.33
DAUNORUBICIN (6)		
25.0	(0.22 ± 0.07)	$a = (6.63 \pm 1.22) \times 10^{-2}$ $S_a = 6.08 \times 10^{-3}$
50.9	(1.73 ± 0.31)	b = -13.35 ± 1.68 S_b = 0.390
76.4	(5.25 ± 1.95)	r = 0.9917 S_y = 0.110
90.0	(12.9 ± 1.5)	

C is an integration constant related to the induction time and k_{obs} is the observed rate constant of degradation reaction.

Either for the interpretation of the straight line plots from ln $c_t/(c_0 - c_t)$ = f(t) or ln c_t = f(t) the following statistical parameters of the equations were calculated by using the least squares method: y = ax + b, $a \pm \Delta a$, $b \pm \Delta b$, standard deviations *Sa, Sb, Sy* and the coefficient of linear correlation r. The values of Δa and Δb were obtained for f = n – 2 degrees of freedom, with α value = 0.05.

The values of reaction rate constants k_{obs} were used to calculate the Arrhenius relationship in order to interpret the influence of the temperature on the reaction rate at 76.4% RH (Fig. 6). The energy of activation and the thermodynamic parameters, enthalpy and entropy of activation for 298 K were calculated based on the parameters of the slope ln k_i = f(1/T) (Table 1).

CONCLUSIONS

The degradation of MMD is an autocatalytic reaction of the first order with respect to MMD concentration at increased temperature and relative air humidity, whereas at an increased temperature and

relative air humidity RH = 0% it is a first-order reaction. Increased relative air humidity is a significant factor determining MMD degradation in solid state. The study has demonstrated that the differences in the chemical structures of MMD and daunorubicin influence their stability, but it does not influence the kinetic mechanism of their degradation. Daunorubicin is more stable in solid state than MMD, but MMD has better pharmacological properties, especially it is less cardiotoxic than daunorubicin.

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