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Influence of Temperature and pH on the Stability of Dimethoxy Biphenyl Monocarboxylate·HCl Solutions

Woo-Chang Choi, Dae-Duk Kim, Young-Hee Shin[1], and Chi-Ho Lee

College of Pharmacy, Pusan national University, Pusan 609-735, Korea and [1]College of Pharmacy, Kyungsung University, Pusan 608-736, Korea

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The accelerated stability of dimethoxy biphenyl monocarboxylate · HCl (DDB-S) was investigated in 6 mg/mL water solution in the pH ranging 2-10 and the temperature of 45-85°C. The observed rate of degradation followed first-order kinetics. The energy of activation for DDB-S degradation was calculated to be 14.1 and 16.5 Kcal/mole at pH 5 and in distilled water, respectively. The degradation rate constant ($K_{25°C}$) obtained by trending line analysis of Arrhenius plots for DDB-S was $5.3 \times 10^{-6} h^{-1}$. The times to degrade 10% (t_{10}) and 50% (t_{50}) at 25°C were 829 and 5,416 days, respectively. DDB-S exhibited the fastest degradation at pH 10 and the slowest rate at pH 5. In addition, at 65°C, degradation rate constants of DDB-S were 0.066, 0.059, 5.460, 32.171, and $1.41 \times 10^{-6} h^{-1}$ at pH 2, 5, 8, 10 and in distilled water, respectively. These observations indicated that the rate-pH profile of DDB-S showed *general acid-base catalysis reaction* in the range of pH 2-10.

Key words: DDB-S, Stability, Self-life

INTRODUCTION

Dimethoxy dimethylene biphenylate (DDB) is a synthetic analogue of Schisandrin C which has been isolated from Fructus Schisandrae, a traditional Chinese herbal medicine (Xie *et al.*,1981). It was found to be effective in improving liver functons and the symptoms of patients with chronic viral hepatitis B (Liu, 1997). Bao *et al.* (1974) demonstrated that the alcoholic extract of the kernels of Fructus Schizandrae protected mice against CCl_4 induced hepatotoxicity. After that, Chen *et al*, (1976) isolated 7 schisandrins from the kernels of Fructus Schizandrae and Xie *et al.* (1981) synthesized Schisandrin C in 1974. DDB was obtained as an intermediate for the synthesis of the regioisomer of Schisandrin C. It was unexpectably shown DDB to protect against CCl_4, D-galactosamine and thioacetamide induced liver injuries in mice and rats (Liu *et al.*, 1979; 1982a; 1982b). Since 1977, DDB has been clinically used in viral hepatitis B patients; it was found that impaired liver functions such as the elevated SGOT or bilirubin and relieved symptoms of the patients improved significantly (Liu Gentago, 1987). In Korea, a prospective randomized controlled trial of a long term therapy with DDB was carried out in chronic active hepatitis (CAH) patients. The results showed that DDB decreased an elevated levels of serum alanine transferase (ALT) and no significant adverse effects were observed except for transient and mild upper gastrointestinal symptoms during DDB treatment (Lee *et al.*, 1991). By these findings, DDB is currently employed as an agent against viral and chemically induced hepatic injury in Korea.

DDB is extremely insoluble in water and, therefore, poorly absorbed from gastro-intestinal (GI) tract after oral administration (i.e., bioavailability is 30%, Liu Gentago, 1987). Despite the application of modern pharmaceutical interventions (Liu Gentago, 1987; Gu *et al.*, 1989; Gu *et al.*, 1990; Hyun and Chun 1991), the oral bioavailability of the drug has not been improved. By these reasons, there has been a great need for parentheral formulation of the drug. Unfortunately, the solubilization of DDB has not been successful.

A new water-soluble analog of DDB, DDB-S (Fig. 1), was designed and synthesized in our laboratory to realize injectable formulation for the use in acute or chronic hepatitis (Lee *et al.*, 1999). In this study, we carried out stability testing of DDB-S since this information is essential in development-preformulation of any new drug. Therefore, the stability of DDB-S in different conditions was studied using HPLC detection method and the

Correspondence to: Chi Ho Lee, College of Pharmacy, Pusan National University, Jangjeon-Dong, Geumjeong-Gu, Pusan 609-735, Korea
E-mail: chiho@hyowon.pusan.ac.kr

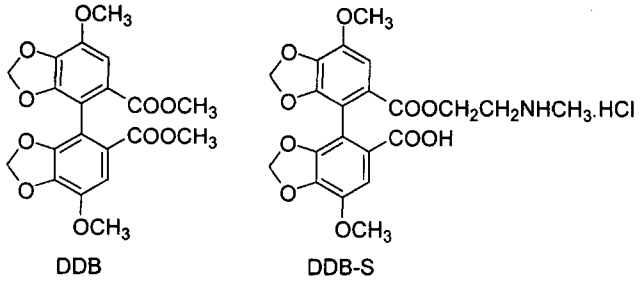

Fig. 1. Structures of DDB and DDB-S

stability kinetics and the degradation rates of DDB-S were estimated by classical chemical methods. [The results of these studies is likely to provide the basis for the reproducible manufacture and storage of injectable DDB-S solutions and its effective use in the treatment of hepatitis.]

MATERIALS AND METHODS

Materials and instruments

DDB was kindly given by Daewoo Pharmaceutical Ind. Co.(Pusan, Korea). DDB-S was synthesized from DDB according to the general method (Lee *et al.*, 1999). HPLC-grade acetonitrile, acetic acid and TLC plate (silicagel 60 F254) were obtained from Merck (Darmstadt, Germany). Ethanol and ether were reagent grade (Hayman Co., England). Testosterone, potassium phosphate monobasic, sodium hydroxide, sodium chloride and boric acid were obtained from Sigma Chemical Co. (St Louis, MO). All other chemicals were reagent grade. Water was prepared with a Millipore Milli-Q Plus device (Millipore, Bedford, MA).

High performance liquid chromatograph (Waters, LC Module I plus) was consisted of Waters 590 programmable pump, Waters 486 Turnable UV detector, Waters 745B data module, in-line degasser and autoinjector. The model S constant temperature water bath (Haake, Germany) was used in the kinetic study. Microprocessor pH meter (HI 9321, Hanna Inst., Portugal) was used for pH measurement of buffer solutions.

Solutions

In this study, 0.2 M HCl-0.2 M KCl (pH 1), 0.2 M acetic acid-0.2 M HCl (pH 3), 0.2 M sodium acetate-0.2 M acetic acid (pH 4, 5), 0.2 M KH_2PO_4-0.2 M NaOH (pH 6, 7, 8) and borate (pH 9, 10) buffers were used. The ionic strength of all buffers was adjusted to 0.5 M by the addition of sodium chloride. Approximately 50 mg testosterone, an internal standard for DDB-S, was weighed accurately, dissolved in 10 mL methanol, and then diluted to 100 mL with mobile phase (see below).

HPLC analysis of DDB-S

The concentration of DDB-S remaining in incubation solutions was determined using a reversed-phase HPLC method. The separation of the compound was carried out using a μ-Bondapak C_{18} column (30 cm, particle size 5 μm, Waters, MA). The mobile phase consisted 0.2 M phosphate buffer (pH 3.0)-acetonitrile (70:30), and the flow rate was 1.0 mL/min. Before analysis, one millilitre of testosterone methanol solution was added accurately to the samples of DDB-S for the HPLC analysis. The injection volume for each sample was 10 μL and the effluent was monitored at 260 nm. Under these conditions, the retention time of DDB-S and testosterone were 5.9 min and 12 min, respectively. DDB-S loss in the buffer solution was monitored as a function of time.

The standard solutions, containing 1, 5, 10, 15 or 20 μg/mL DDB-S and 5 μg/mL testosterone, were prepared in acetonitrile-0.2 M phosphate buffer (30:70). An aliquot of the standard solution was injected on the chromatograph for DDB-S calibration.

Kinetic study for DDB-S

Approximately 30 mg DDB-S was weighed accurately, dissolved in 5 mL water or pH 5 acetate buffer solution, and then diluted to 300 mL with water or acetate buffer to obtain a concentration of 100 μg/mL. A 3 mL aliquot of the solution was added to 5 mL amber glass ampoules, which were flame sealed and maintained in a constant temperature bath in which the appropriate temperature (Table I) had been adjusted in a light-protected environment. Ampoules were withdrawn from the water bath at suitable time intervals and immediately analysed. All analyses were carried out in triplicate. In addition, the temperature dependence of DDB-S stability was conducted at 45, 65 and 85°C at pH 5 and in distilled water. The degradation rate constants were estimated the temporal profile of DDB-S levels under various incubation temperatures.

When it was necessary to estimate the dependency of the kinetics of degradation on the temperature, log k was plotted against the reciprocal of absolute temperature (1/T) and the activation energy was calculated from the slope.

Table I. Rate constants and activation energies for DDB-S degradation at pH 5 buffer and in distilled wate

Solution	Temp. (°C)	[a]k × 10^{-4} (day^{-1})	Activation energies (kcal/mole)	Arrhenius factor (h^{-1})
Distilled water	45	1.67 ± 0.21		
	65	141.62 ± 5.21	16.5	7.74×10^{20}
	85	892.05 ± 17.32		
pH 5 buffer	45	0.43 ± 0.02		
	65	5.92 ± 0.62	14.1	5.01×10^{16}
	85	98.11 ± 6.60		

[a]Data is expressed as mean ± s.d. for n=3

Dependency of the kinetics of DDB-S degradation on pH

Fifty μL aliquots of DDB-S solution (concentration 10 mg/mL, in water) were added to amber ampoules containing 5 mL of buffer solutions with pH ranging from 2 to 10. The ampoules were sealed by fusion. Twenty seven samples (i.e., triplicate for each pH) were prepared and triplicate analysis were carried out for each pH. All samples were maintained at 65°C in constant temperature water bath until the HPLC analysis for remaining DDB-S.

RESULTS AND DISCUSSION

Quantification of DDB-S

When plotted against the concentration, the peak area for DDB-S was linearly correlated with concentrations in the range of 1.0-20 μg/mL (data not shown). The correlation coefficients was found to be 0.9999, indicating a good linearity.

Effect of temperature on DDB-S drgradation

The effect of temperature on kinetics of DDB-S degradation was studied in pH 5 buffer solution and in distilled water, by measuring the degradation rates at 45, 65 and 85°C. As expected, the degradation of DDB-S was temperature dependent; An increase in the temperature resulted in an increase in the rate of degradation at both of pH 5 buffer and distilled water. In addition, temporal profiles of % remaining DDB-S (Fig. 2) appear consistent with the first-order degradation kinetics for DDB-S in the temperature range (45-85°C) used in this study. To determine the temperature effect quantitatively, log k values were plotted against the reciprocal of absolute temperature (1/T) (i.e., an Arrhenius plot for DDB-S, Fig. 3), resulting in an estimation of E_a (kcal/mole) for DDB-S (Table I). The fact that E_a was independent of temperature suggest that the degradation mechanism of DDB-S did not change with temperature. Extrapolation of the regression line to 25°C resulted the $k_{25°C}$ of DDB-S to be $5.3 \times 10^{-6} h^{-1}$, indicating that times to degrade 10% (t_{10}) and 50% (t_{50}) at 25°C were 829 and 5,416 days, respectively.

Dependency of DDB-S degradation on pH

In order to determine the stable pH region for DDB-S in aqueous solutions, the stability of DDB-S was evaluated under various pH conditions at 65°C. Temporal profiles of percent remaining DDB-S at various pHs were shown in Fig. 4. The rate constants at 65°C were calculated from the slopes for each pH and listed in Table II. Comparison of rates indicated that the degradation was the fastest for the most basic condition (i.e., pH 10, k, $0.3217\ h^{-1}$)) while the slowest degradation was found at pH 5 (k, $5.92 \times 10^{-4} h^{-1}$). Thus, the results showed that DDB-S was appeared to be reasonably stable in the pH ranges between 2 and 5. In addition, the degradation was significantly accelerated in basic pH's.

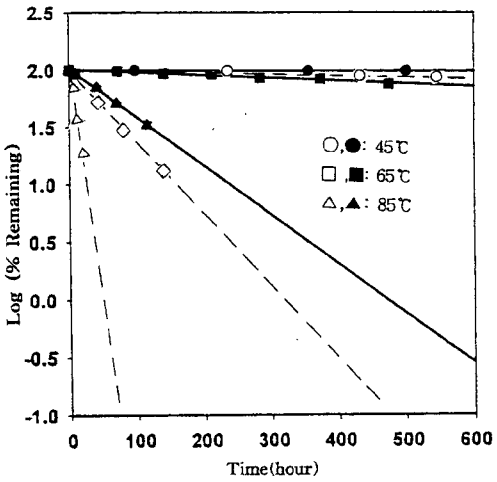

Fig. 2. Effect of temperature on percent DDB-S remaining in pH 5 buffer (---) and in distilled water(– –) solutions at different time points

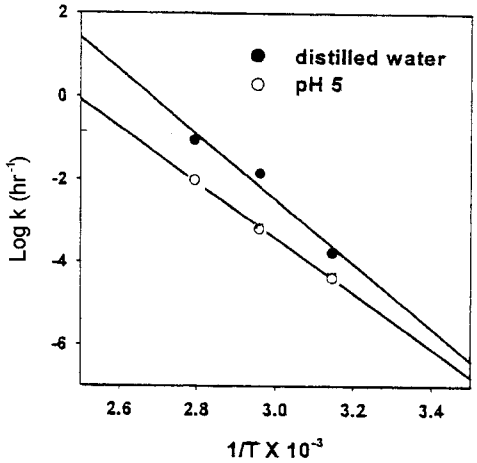

Fig. 3. Arrhenius plot of DDB-S degradation

Table II. Effect of various pH conditions on the rate constant for DDB-S degradation at 65°C

pH	[a]Rate constant ($10^{-4} h^{-1}$)
2	6.56 ± 0.35
3	9.72 ± 0.22
4	6.08 ± 0.46
5	5.92 ± 0.44
6	44.61 ± 6.60
7	348.47 ± 17.35
8	546.55 ± 23.67
9	1253.76 ± 44.56
10	3217.38 ± 75.83

[a]Data is expressed as means ± .d. for n=3

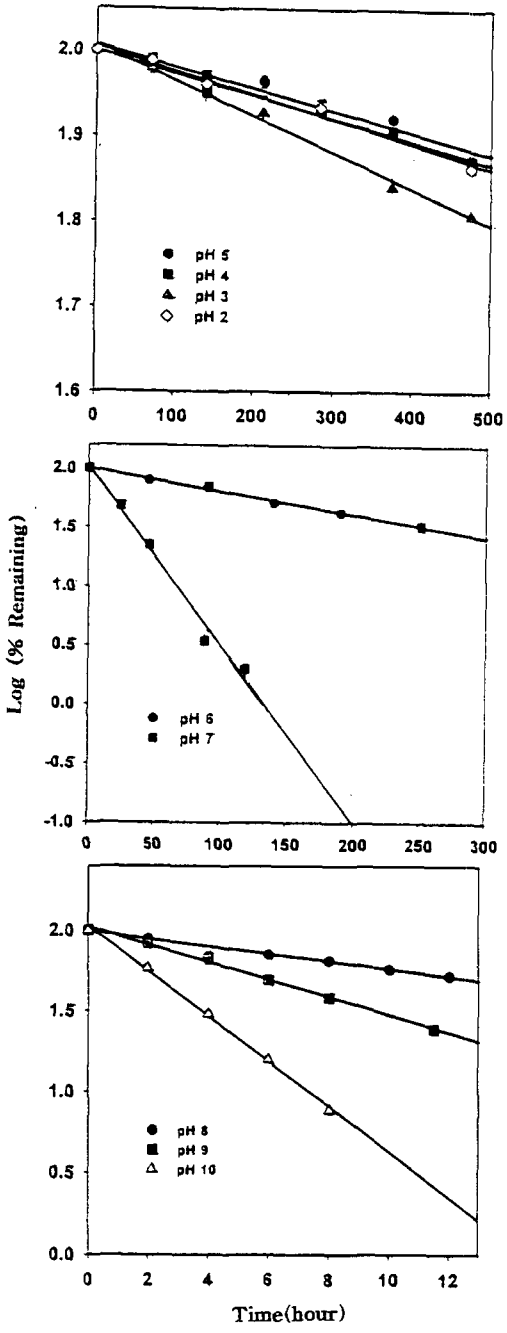

Fig. 4. The effect of pH on DDB-S stability at 65°C

The profile for log k versus pH for the degradation of DDB-S was shown in Fig. 5. The profile is consistent with the hypothesis that DDB-S degradation is mediated by the general acid-base catalysis; a decrease in rate with the increasing pH from 2 to 5 may be mediated by general acid catalysis, while a further increase in the degradation rate in pH from 5 to 10 may be governed by general base catalysis mechanism. These observation indicated that one or more species of the buffer components may be involved in the degradation of DDB-S. It is noted

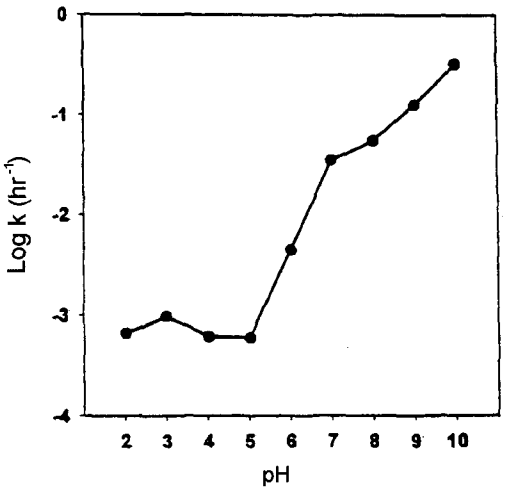

Fig. 5. Rate-pH profile for the degradation of DDB-S at 65°C

that, near pH 5, a minimum rate constant was observed and, thus, the optimal formulation pH for DDB-S solution is pH 5.

CONCLUSION

In this study, DDB-S found to be more stable in a slightly acidic pH than in distilled water or an alkalic environment. The degradation appear to follow the first-order kinetics, which is accelerated with temperature. The linearity of Arrhenius plots indicated that the reaction mechanism was independent of the temperature. In addition, rate-pH profile of the degradation suggested the general acid-base catalysis degradation for DDB-S. Therefore, these observations indicated that the storage of DDB-S solution in slightly acidic pH with a lowered temperature may be necessary for an extended stability of the compound.

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