Study on the Hydrolysis of 2-Chlorobenzamide

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It is reported that 2-chlorobenzamide, one of the chief degradation products of CCU (1-(2-chlorobenzoyl)-3-(4-chlorophenyl) urea), a new insect growth regulator, is a potential carcinogen, but few studies about its environmental stability have been found. This paper is concerned with the hydrolysis of 2-chlorobenzamide as part of the environmental study of CCU. The results showed that 2-chlorobenzamide is relatively stable in solutions of pH = 6 and 8, for which the rate constants are 0.00286 h^{-1} (R = 99.13%, SD = 0.0095) and 0.00109 h^{-1} (R = 96.70%, SD = 0.0072), respectively. Hydrolysis was more rapid in acidic (pH = 5), alkaline (pH = 10), and neutral (pH = 7) environments, with hydrolytic rate constants of 0.00417h^{-1} (R = 95.76%, SD = 0.0390), 0.00411h^{-1} (R = 99.89%, SD = 0.0162) and 0.00408h^{-1} (R = 98.29%, SD = 0.0237), respectively. The change of the rate of hydrolysis with pH showed two minima at 25 °C. Temperature has some impact on the hydrolysis, showing at higher temperature the larger rate of reaction.

Keywords: *Hydrolysis; 2-chlorobenzamide; CCU (1-(2-chlorobenzoyl)-3-(4-chlorophenyl) urea)*

1. INTRODUCTION

CCU is an insecticide that disrupts normal moulting and development processes of insects, similar to DFB and PH-6038, two benzoyl phenylurea compounds. It was synthesized and commercialized in China, where it was introduced to apply to large acreages in agriculture and forestry as a new, potent insecticide. In the future it may be used widely in the other countries. The degradation behavior of CCU in the environment had been systematically studied and reported (Liu et al., 1990, 1992; Liu and Xu, 1993a; Shi, 1990; Yang et al., 1992, 1993). CCU may degrade via hydrolysis, biodegradation, and photodegradation depending on a variety of environmental factors. 2-Chlorobenzamide is a major degradation product of CCU. A study of DNA-2-chlorobenzamide adduct formation has been carried out (Liu et al. 1997), and the result was positive. The Ames assay of 2-chlorobenzamide was positive also.

These results indicate that 2-chlorobenzamide is a potential carcinogen. Because CCU basically has no biotoxicity on nontarget organisms (Liu and Xu, 1993b), the environmental effect of CCU is chiefly due to the behavior of its major degradation products, and 2-chlorobenzamide should be given more attention owing to its potential carcinogenicity. But few prior reports about the environmental behavior of 2-chlorobenzamide exist. This paper describes the hydrolysis of 2-chlorobenzamide, as part of a systematic evaluation of the environmental safety of CCU.

2. MATERIALS AND METHODS

2.1. Parameters and Chemicals. A high-performance liquid chromatograph (HPLC) with Shimadzu LC-6A (Japan), Shimadzu SPD-6AV UV−vis spectrophotometric detector, and 25 cm × 4.6 mm ZORBAX-ODS reversed-phase column was used for all quantitative analyses.

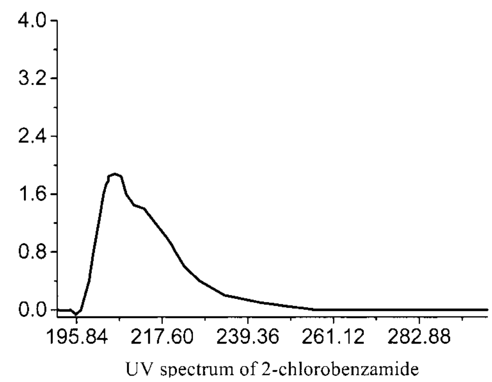

Figure 1.

2-Chlorobenzamide was obtained from the Research Center of Eco-Eenvironmental Sciences, Chinese Academy of Sciences, Beijing. It had no impurity peaks by HPLC.

2.2. Preparation of the Buffer Solutions. pH = 5.00 solutions of 238 mL of 0.1 M NaOH and 500 mL of 0.1 M $C_6H_4C_2O_4HK$ were mixed and then diluted to 1000 mL.

pH = 6.00 solutions of 125 mL of 0.1 M KH_2PO_4 and 14 mL of 0.1 M NaOH were mixed and then diluted to 250 mL.

pH = 7.00 solutions of 297 mL of 0.1 M NaOH and 500 mL of 0.1 M KH_2PO_4 were mixed and then diluted to 1000 mL.

pH = 8.00 solutions of 125 mL of 0.1 M KH_2PO_4 and 116.5 mL of 0.1 M NaOH were mixed and then diluted to 250 mL.

pH = 9.00 solutions of 125 mL of 0.025 M $Na_2B_4O_7$ and 11.5 mL of 0.1 M HCl were mixed and then diluted to 250 mL.

pH = 10.00 solutions of 291 mL of 0.2 M H_3BO_3 and 709 mL of 0.2 M KCl (0.2 M Na_2CO_3) were mixed and then diluted to 1000 mL.

Twice distilled water was used for all dilutions. All of the pH values of the buffer solutions were measured by a pH meter (pHs-2, China) and adjusted to the needed value ± 0.02.

2.3. HPLC Conditions. The UV spectrum of 2-chlorobenzamide showed a maximum absorbance at 206 nm (see Figure 1), so 206 nm was chosen as the detection wavelength for HPLC. Four different methanol and water mixtures were tested as the HPLC mobile phase (methanol/water (v/v) = 8:2;

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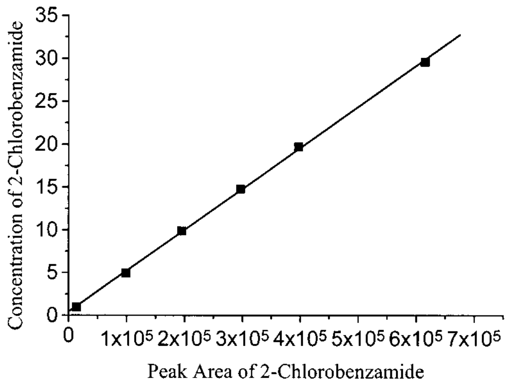

Figure 2.

7:3; 6:4; 4:6). The retention times of 2-chlorobenzamide with these mobile phases at a flow rate of 1 mL/min at 32 °C were 3.7, 3.9, 4.3, and 6.3 min, respectively. The mixture 40% methanol−60% water at a flow rate of 1 mL/min was chosen to avoid the interference of solvent methanol whose retention time was 2.2−3.4 min.

2.4. Working Curve. A series of solutions were prepared as 0.986, 4.93, 9.86, 14.79, 19.72, and 29.58 ppm by diluting a 493 ppm 2-chlorobenzamide stock solution. These solutions were analyzed by HPLC with 5 μL of solution as injection. The resulting calibration line had a linear relationship from 0.986 to 29.58 ppm, with a correlation coefficient of 0.9998 (Figure 2). Following the equation:

$$C = 4.781 \times 10^{-5}A + 0.448$$

2.5. Recovery of 2-Chlorobenzamide. Concentrations of 0.493, 0.789, and 0.986 ppm of 2-chlorobenzamide in twice-distilled water were used to study the relationship between recovery and concentration. Also, 0.5 mL of 493 ppm 2-chlorobenzamide standard solution was added to 150 mL buffer solutions at pH = 5, 7, and 10 to study the relationship of recovery and pH of buffer solution. Aliquots of 10 mL were taken out at several times, and each sample was extracted with 3 × 10 mL of freshly distilled dichloromethane. The extracts were combined and dried over anhydrous Na_2SO_4 and then evaporated to near dryness in a rotary vacuum evaporator. The residues were dissolved in 1 mL freshly distilled anhydrous methanol and analyzed by HPLC.

Recoveries of 2-chlorobenzamide with concentrations of 0.493, 0.78, and 0.986 ppm were 88.8 ± 1.20%, 93.2 ± 1.32%, and 96.8 ± 0.95%, respectively, and recoveries of 2-chlorobenzamide with buffer solutions at pH = 5, 7, and 10 were 91.2 ± 0.80%, 90.8 ± 1.45%, and 94.5 ± 1.26%, respectively.

2.6. Effect of pH on the Hydrolysis of 2-Chlorobenzamide. Six samples of 150 mL of various buffer solutions (pH = 5, 6, 7, 8, 9, and 10) were fortified with 0.5 mL of 2-chlorobenzamide stock solution by vigorously shaking. These samples were then maintained at 25 °C and covered with a black shade to avoid light. At 12 h intervals, 10 mL duplicate samples were taken and extracted with dichloromethane. Extraction was carried out, and the variation of concentration of 2-chlorobenzamide in buffer solutions with time was studied as described above.

2.7. Effect of Temperature on the Hydrolysis of 2-Chlorobenzamide. The effect of temperature on the hydrolysis of 2-chlorobenzamide in three different buffer solutions (pH = 5, 7, and 10) was studied at 25, 35, 45, and 55 °C. The container containing 150 mL of buffer solution fortified with 0.5 mL of stock solution of 2-chlorobenzamide was set at a certain temperature in the thermostat. At several time intervals, 10 mL duplicate samples were taken out, extracted with dichloromthane, and analyzed by HPLC to obtain the variation of concentration of 2-chlorobenzamide with time.

3. RESULTS AND DISCUSSION

3.1. Effect of pH on the Hydrolysis of 2-Chlorobenzamide. The effect of pH on the hydrolysis of 2-chlorobenzamide is shown in Figure 3. Throughout the hydrolysis of 2-chlorobenzamide at 25 °C, there was an excellent linear relationship between ln c (concentration of 2-chlorobenzamide) and t (time). These indicated that the hydrolysis of 2-chlorobenzamide is of the first order. The linear regression equations are as follows:

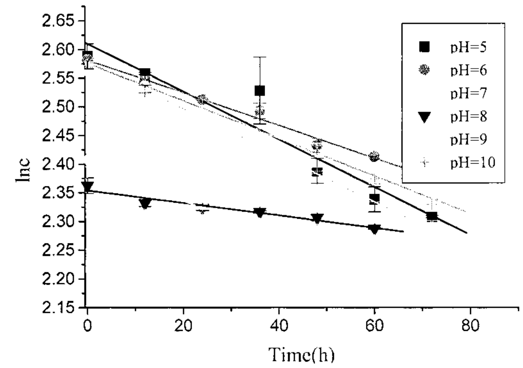

Figure 3. Hydrolytic behavior of 2-chlorobenzamide at 25 °C.

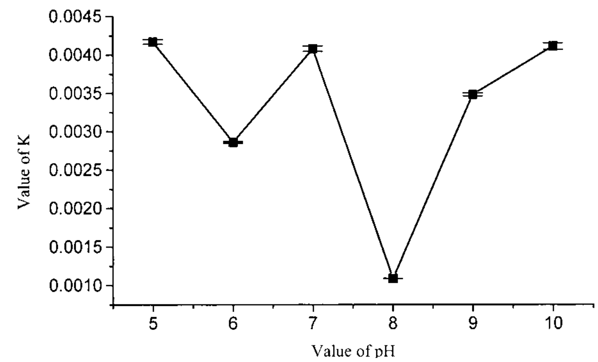

Figure 4. Effect of pH on the hydrolysis of 2-chlorobenzamide.

$$\ln c = 2.61016 - 0.00417t \quad (R = 95.76\%, \text{SD} = 0.0390)\ \text{pH} = 5$$

$$\ln c = 2.58175 - 0.00286t \quad (R = 99.13\%, \text{SD} = 0.0095)\ \text{pH} = 6$$

$$\ln c = 2.57852 - 0.00408t \quad (R = 98.29\%, \text{SD} = 0.0237)\ \text{pH} = 7$$

$$\ln c = 2.35398 - 0.00109t \quad (R = 96.70\%, \text{SD} = 0.0072)\ \text{pH} = 8$$

$$\ln c = 2.40589 - 0.00348t \quad (R = 98.77\%, \text{SD} = 0.0139)\ \text{pH} = 9$$

$$\ln c = 2.57479 - 0.00411t \quad (R = 99.89\%, \text{SD} = 0.0162)\ \text{pH} = 10$$

The half times of degradation ($t_{1/2}$) were 166.2, 242.4, 169.4, 635.9, 199.2, and 168.6 h, respectively, at pH = 5, 6, 7, 8, 9, and 10. The influence of pH on the hydrolysis of 2-chlorobenzamide is shown in Figure 4. The reaction rate (k) variation with pH showed two minima, and the concentration of 2-chlorobenzamide declined relatively quickly in acid (pH = 5), alkaline (pH = 10), and neutral (pH = 7) conditions, but it declined more slowly at pH = 6 and 8. Thus, 2-chlorobenzamide was less stable in acid, alkaline, and neutral environments but relatively more stable at pH = 6 and 8.

There are two function groups −C=O and $-NH_2$ in amide molecule, which lead to the complex mechanisms of amide hydrolysis. Two mechanisms, acid-catalyzed and base-catalyzed mechanisms, are involved in amide hydrolysis. Now it is generally agreed that the hydrolysis reaction of amide begins with protonation on the amide oxygen, followed by water attack at carnbonyl carbon (Solomons, 1996; March, 1992; Lowry et al., 1987; Isaacs, 1987). Some workers believe that alternate routes do exist in which water directly displaced an amine molecule from the N-protonated amide (Smith and Yates, 1972). In the opinion of Robinson (Robinson and Tester, 1990), the alkaline hydrolysis mechanism is appropriate for the hydrolysis of amide. According to our experimental results, we suggest that the hydrolysis of 2-chlorobenzamide is catalyzed by H^+ or OH^-; at the same time, protonation on the amide oxygen competes with protonation on the amide nitrogen in the process of 2-chlorobenzamide hydrolysis. All these resulted in the complexity of the hydrolysis of 2-chlorobenzamide. So the effect of pH on the hydrolysis of 2-chlorobenzamide is complex like the results of our experiment.

3.2. Effect of Temperature on the Hydrolysis of 2-Chlorobenzamide. Three groups of experiments were carried out to study the effect of temperature on the hydrolysis of 2-chlorobenzamide under different buffer solutions (pH = 5, 7, and 10). In all cases, the hydrolysis rate of 2-chlorobenzamide increased with temperature (see Figures 5–8). The linear regression equations are as follows:

pH = 5

$$\ln c = 2.61016 - 0.00417t \quad (R = 95.76\%, \mathrm{SD} = 0.0390, 25\ ^\circ\mathrm{C})$$

$$\ln c = 2.64400 - 0.00587t \quad (R = 98.58\%, \mathrm{SD} = 0.0250, 35\ ^\circ\mathrm{C})$$

$$\ln c = 2.46479 - 0.00598t \quad (R = 99.30\%, \mathrm{SD} = 0.0091, 45\ ^\circ\mathrm{C})$$

$$\ln c = 2.29515 - 0.00805t \quad (R = 99.50\%, \mathrm{SD} = 0.0169, 55\ ^\circ\mathrm{C})$$

pH = 7

$$\ln c = 2.57852 - 0.00408t \quad (R = 98.29\%, \mathrm{SD} = 0.0237, 25\ ^\circ\mathrm{C})$$

$$\ln c = 2.74395 - 0.00553t \quad (R = 93.77\%, \mathrm{SD} = 0.0300, 35\ ^\circ\mathrm{C})$$

$$\ln c = 2.53688 - 0.00469t \quad (R = 97.87\%, \mathrm{SD} = 0.0247, 45\ ^\circ\mathrm{C})$$

$$\ln c = 2.33972 - 0.00535t \quad (R = 99.12\%, \mathrm{SD} = 0.0121, 55\ ^\circ\mathrm{C})$$

pH = 10

$$\ln c = 2.57479 - 0.00411t \quad (R = 99.89\%, \mathrm{SD} = 0.0162, 25\ ^\circ\mathrm{C})$$

$$\ln c = 2.62251 - 0.00332t \quad (R = 99.08\%, \mathrm{SD} = 0.0114, 35\ ^\circ\mathrm{C})$$

$$\ln c = 2.44791 - 0.00415t \quad (R = 97.14\%, \mathrm{SD} = 0.0250, 45\ ^\circ\mathrm{C})$$

$$\ln c = 2.45413 - 0.00433t \quad (R = 98.56\%, \mathrm{SD} = 0.0098, 55\ ^\circ\mathrm{C})$$

The effect of temperature is helpful to the hydrolysis of 2-chlorobenzamide. However, the hydrolysis appeared

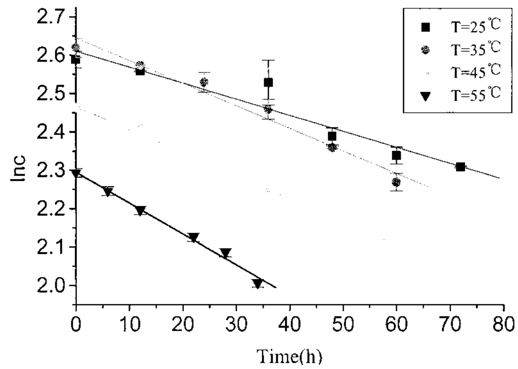

Figure 5. Effect of T on the hydrolysis of 2-chlorobenzamide (pH = 5).

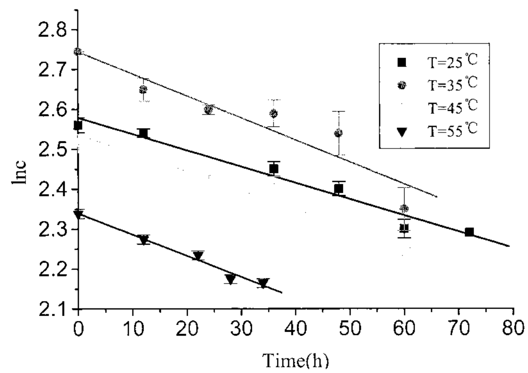

Figure 6. Effect of T on the hydrolysis of 2-chlorobenzamide (pH = 7).

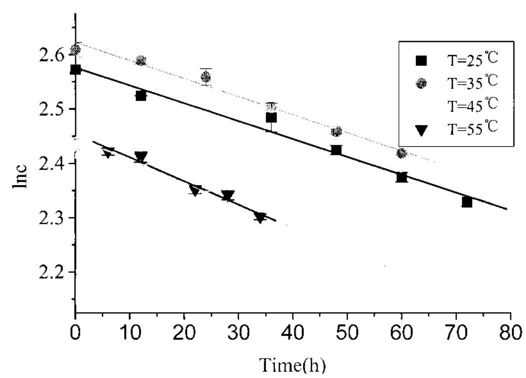

Figure 7. Effect of T on the hydrolysis of 2-chlorobenzamide (pH = 10).

a special value at 35 °C in the pH = 7, 10 solutions. In pH = 7 solution the rate of hydrolysis at 35 °C is higher than those at other temperatures, but in pH = 10 solution the rate of hydrolysis at 35 °C is much smaller than those at other temperatures. The reasons that led to this result are unknown and require further study.

From the experimental results, we can see that 2-chlorobenzamide will hydrolyze if it is put into water, but the hydrolysis rate constant is different in different pHs and different temperatures. At pH = 8, the half time of 2-chlorobenzamide is the longest at 635.9 h. This means that 2-chlorobenzamide is relatively stable in water in this condition. When CCU was applied in water or field, it will hydrolyze and produce 2-chlorobenzamide. Due to 2-chlorobenzamide's potential carcinoge-

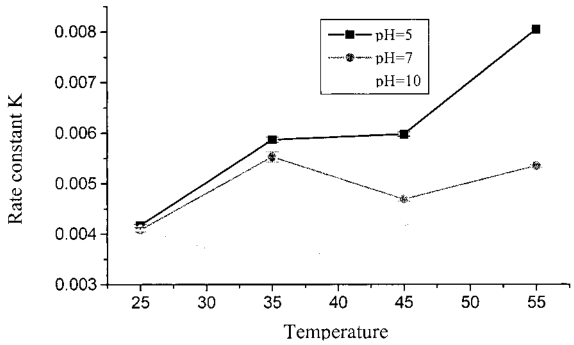

Figure 8. Relationship between K and T.

nicity, a caution is warranted for the application of CCU in or near water.

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