Bull. Environm. Contam. Toxicol. 23,456-463 (1979)

Reactions of Aqueous Chlorine Dioxide with Amino Acids Found in Water

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Amino acids are known to be present in free or combined form in water (CROLL 1972). Although they are essential for growth, the products that they could produce during the disinfection of water might be harmful. Reactions of chlorine with amino acids are reported to be oxidative decarboxylation, producing aldehydes and nitriles, and chlorine substitution giving rise to chlorinated amino acids (GILBERT 1978). Chlorine dioxide is used for taste and odour control and has been suggested as an alternative to chlorine in water treatment facilities. It is, therefore, important to identify the products formed during chlorine dioxide treatment of raw water supplies.

There are several reports on the reactions of amino acids with chlorine dioxide. Chlorine dioxide oxidation of tryptophan gave isatin, indoxyl, pink indigo red and unidentified yellow orange substances (FUJII and UKITA 1957). Tyrosine has been reported to give pink dopaquinone and red dopachrome and this reaction has been used in the colorimetric determination of chlorine dioxide (HODGEN and INGOLS 1954). Cystine was also found to be reactive towards chlorine dioxide and the products were believed to be fragmented organic sulfonic acids produced by sulfur-sulfur bond cleavage and oxidation (GILBERT 1978, SCHMIDT and BRAUNSDORF 1922). It was found (KENNAUGH 1957) that a 50% solution of glacial acetic acid saturated with chlorine dioxide (diaphanol) reacted with numerous amino acids and the disappearance of the amino acids was monitored by the ninhydrin reaction. Phenylalanine was broken down to alanine after eighteen hours and no ninhydrin positive material was present after three days.

On the other hand it was reported that phenylalanine and some other amino acids were unreactive towards chlorine dioxide for 72 hours, based on the determination of unreacted oxidant (SCHMIDT and BRAUNSDORF 1922). A separate report (BENARDE et al. 1967) states that histidine, asparagine, phenylalanine, arginine, proline and leucine are unreactive when treated with chlorine dioxide for a period of 30 minutes. Palin has assumed that glycine does not react with chlorine dioxide and used glycine to remove chlorine in the titrimetric determination of chlorine dioxide (PALIN 1974).

0007-4861/79/0023-0456 $01.60

It was found during our studies on analytical methods for chlorine dioxide determination that glycine does react with chlorine dioxide (TAYMAZ and WILLIAMS 1979) and we have, therefore, investigated in greater detail the reaction of chlorine dioxide with amino acids in aqueous solution using glycine and phenylalanine as model compounds.

EXPERIMENTAL

Infrared spectra were recorded on a Perkin-Elmer, Model 457, infrared spectrometer using KBr pellets. Nuclear magnetic resonance spectra were determined using a Bruker Instrument Model VP-80. Samples were dissolved in D_2O, DCl was added to increase the solubility of phenylalanine and the internal standard was sodium-2,2-dimethyl-2-silapentane-5-sulfonate (DSS). Gas chromatographic analyses were conducted on a Perkin-Elmer, Model 910, gas chromatograph equipped with a flame ionization detector and using helium as the carrier gas at a flow rate of 60 mL/min. The 1.8 m x 2 mm i.d. coiled glass column was packed with 3% OV-17 on Chromosorb 750, 80-100 mesh. The oven was programmed from 80 to 240° at 5°C/min. The injection and detector temperatures were 240 and 270°C, respectively. The samples were dissolved in methanol for GC analysis.

Gas chromatographic-mass spectrometric analyses were performed on a Finnigan, Model 4000, automated gas chromatograph-mass spectrometer, using the above mentioned column. The carrier gas was helium and had a flow rate of 20 mL/min. The mass spectrometer conditions were: electron impact ionization energy 70 eV, electron multiplier 1400 volts, sensitivity 10E-7, source temperature 290°, separator 240°C.

Colorimetric measurements were made on a Unicam SP 1800 Ultraviolet Spectrophotometer and fluorometric measurements were performed using an Aminco-Bowman spectrophotofluorometer, Model J4-8961. Silica gel thin layer chromatography plates (0.25 mm) were purchased from Analtech Inc. and were developed using; Solvent A, n-butanol:acetic acid:water (60:20:20) (BRENNER and NIEDERWEIESER 1960) or solvent B, ethyl acetate. Spots were visualized by viewing under UV light or by ninhydrin spray reagent (FRODYMA and FREI 1964). A Beckman, Model 121 M Automated Amino Acid analyzer was used for some quantitative determinations.

Glycine, ninhydrin and L-β-phenylalanine were analytical reagent grade and all solvents were glass distilled. Chlorine dioxide free from chlorine was prepared as previously described (TAYMAZ and WILLIAMS 1979).

Reaction of Glycine with Chlorine Dioxide: Glycine (85 mg; 1 mMole) was stirred with chlorine dioxide (134 mg; 2 mMole) in water (100 mL) for 60 hours at 23°C. Nitrogen was then bubbled through the solution to remove excess chlorine dioxide and the mixture was evaporated to dryness at 60°C using a rotary evapo-

rator. The residue was extracted with ether (3 x 30 mL), then with methanol (3 x 30 mL). The combined ether extracts were dried (Na_2SO_4), evaporated and the residue dissolved in hexane and analyzed by TLC, (solvent B), GC and GC-MS. The combined methanol extracts were concentrated to a small volume and analyzed by TLC (solvent A). The solution was further analyzed by GC-MS after formation of N-trifluoroacetylated n-butyl ester derivatives (ROACH and GEHRKE 1964).

Reaction of Phenylalanine with Chlorine Dioxide: Phenylalanine (165 mg; 1 mMole) was treated with chlorine dioxide (134 mg; 2 mMole) in water (100 mL), either at 70°C for 3 hours or at 23°C for 48 hours. The products were isolated and identified in a similar manner to those for glycine.

Ninhydrin Reaction for Amino Acid Quantitation: Glycine (82 mg; 1 mMole) was dissolved in aqueous ClO_2 (100 mL, 1.5 g/L), aliquots (5 mL) were taken at time intervals and unreacted ClO_2 was removed from the aliquot using the nitrogen gas. To each aliquot ninhydrin reagent (0.5 mL) was added and the mixtures were heated at 110° for 20 minutes, with occasional shaking. The absorbance of the samples was then recorded at 570 nm by means of an ultraviolet spectrophotometer (McCALDIN 1960).

Fluorescence Reaction for Amino Acid Quantitation: Glycine (2 to 3 mg) was dissolved in aqueous chlorine dioxide (100 mL; 1.5 g/L), and the mixture was stirred at a controlled temperature. Aliquots (0.5 - 1 mL) were taken at time intervals and ClO_2 was bubbled off with nitrogen. To each aliquot phthalaldehyde reagent was added. The fluorescence was then measured at 455 nm (ROTH 1971).

RESULTS AND DISCUSSION

During an investigation of methods of analysis for chlorine dioxide in aqueous solution (TAYMAZ and WILLIAMS 1979) it was discovered, contrary to previous reports (PALIN 1974), that the chlorine dioxide levels decreased when glycine was used in the titration. In the presence of a five fold molar excess of glycine 50% of the chlorine dioxide had reacted in one hour at room temperature (TAYMAZ and WILLIAMS 1979). Further investigation has now confirmed that the chlorine dioxide reacts with glycine in aqueous solution. The presence of glycine can be monitored either colorimetrically, after reaction with ninhydrin, or fluorometrically, after reaction with phthalaldehyde. It can be seen with the colorimetric method (Fig. 1) that, in the presence of threefold molar excess of chlorine dioxide, at least 35% of the glycine has reacted after 80 min. at room temperature. Using the more sensitive fluorometric method the effect of temperature on the reaction was monitored at 0, 23 and 55°C (Fig. 2) in the presence of excess chlorine dioxide.

At 0°C there appears to be a rapid initial reaction and then no further reaction. At the two higher temperatures there is again

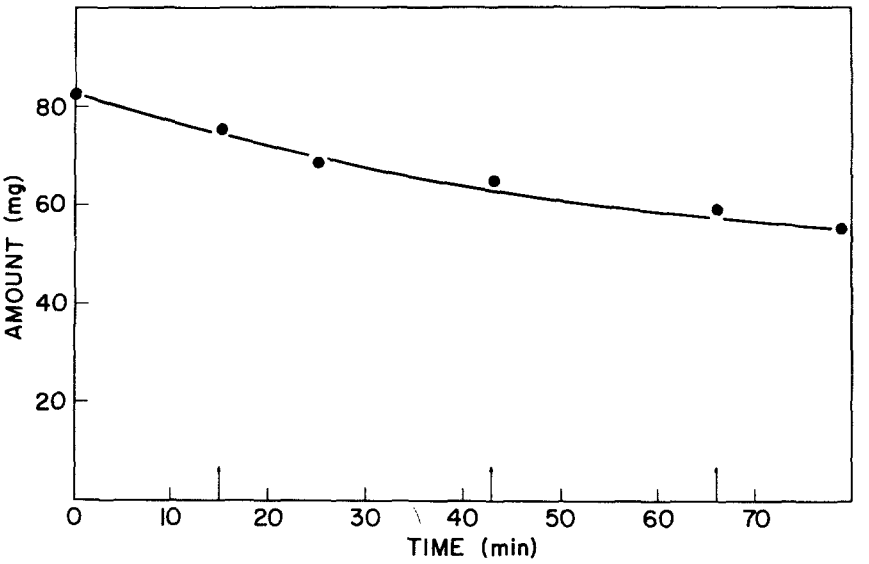

Figure 1. Reaction of glycine with aqueous ClO_2 at 23° as monitored colorimetrically.

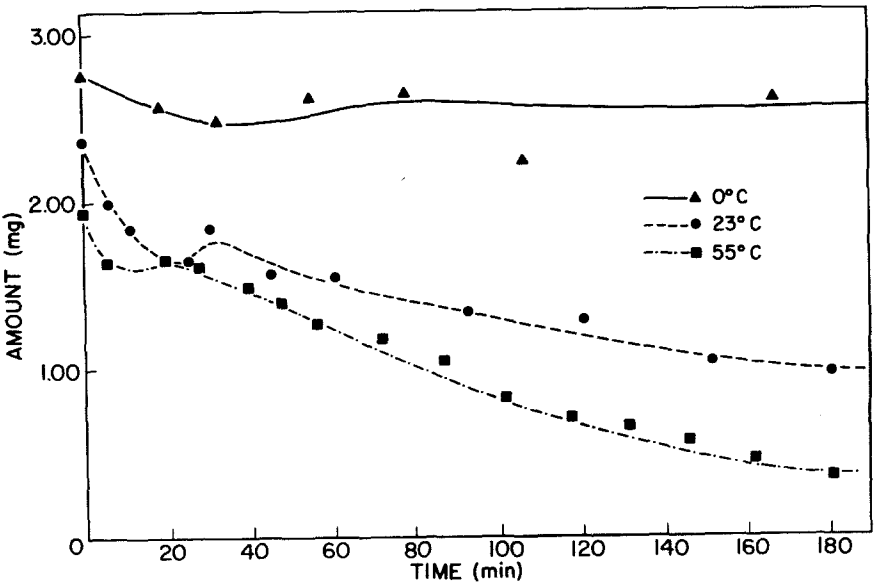

Figure 2. Effect of temperature on the reaction of glycine with aqueous ClO_2 as monitored fluorometrically.

an initial rapid reaction followed by a slower secondary reaction with at least 50% reaction after 80 minutes. Since the fluorometric and colorimetric methods may also measure reaction products as well as glycine (PAVLOVA 1961, ZACHARIUS and TAILLEY 1962, SCHILLING et al. 1963) it is not possible to directly relate the values in Figs. 1 and 2 to actual glycine concentrations. However, it can be clearly seen that glycine does indeed react with chlorine dioxide in aqueous solution.

Analysis of the reaction mixtures from the room temperature reaction by gas chromatography and thin layer chromatography did not reveal any glyoxal, oxalic acid, glycolic acid or glyoxylic acid. Formaldehyde was qualitatively identified by a positive Schiff-Elvove test (WELCHER 1966). By visual estimation of the intensity of colour obtained by this test the levels of formaldehyde appeared to increase and then decrease during the reaction. Carbon dioxide was also detected as a reaction product by passing nitrogen gas through the reaction flask and then through a barium hydroxide solution. The rapid appearance of a fine white precipitate was considered to be a positive test for carbon dioxide since no fine precipitate was obtained from passing chlorine dioxide and nitrogen through barium hydroxide solution.

These findings are consistent with those reported by other workers. Manganese dioxide oxidation of glycine has been reported to produce carbon dioxide and ammonia (BARAKAT et al. 1956) and glycine is also reported to react with hypochlorite to give carbon dioxide and formic acid (NORMAN 1936).

The reactions of phenylalanine and chlorine dioxide were studied next. To evaluate the rate of reaction, phenylalanine was reacted with excess of ClO_2 at 0, 23 and 55°C and the reaction was monitored fluorometrically, and the results are shown in Fig. 3. At 0°C and 23°C there appears to be a fast reaction(s) followed by a slow secondary reaction(s) while at 55°C all reactions appear to be fairly rapid. Again because the fluorometric method may also measure reaction products as well as phenylalanine it is not possible to directly relate values in Fig. 3 to actual phenylalanine concentrations. However, it is obvious that chlorine dioxide does react with phenylalanine in aqueous conditions.

In order to identify reaction products a preparative scale reaction was carried out with a two molar excess of chlorine dioxide. Since the rate of reaction was extremely slow at room temperature the reaction was carried out at 70° and was monitored by the ninhydrin reaction (Fig. 4) on aliquots of the reaction mixture. It can be seen that there was an initial decrease in ninhydrin positive materials followed by an increase as secondary reactions occurred and then a gradual decrease in ninhydrin positive materials. After 3 hours the reaction was stopped, excess chlorine dioxide removed and the reaction mixture concentrated to dryness. TLC analysis (Solvent A) of a portion of the residue

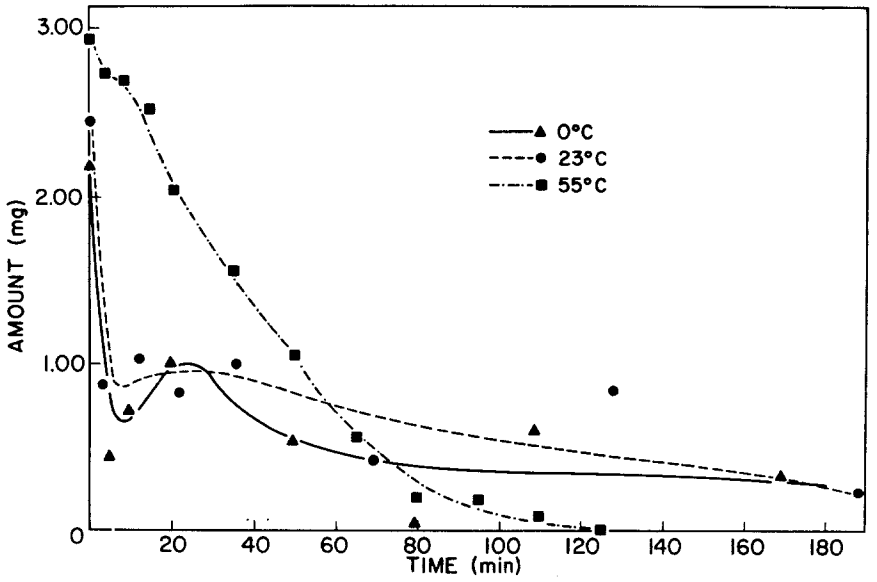

Figure 3. Effect of temperature on the reaction of phenylalanine with aqueous ClO_2 as monitored fluorometrically.

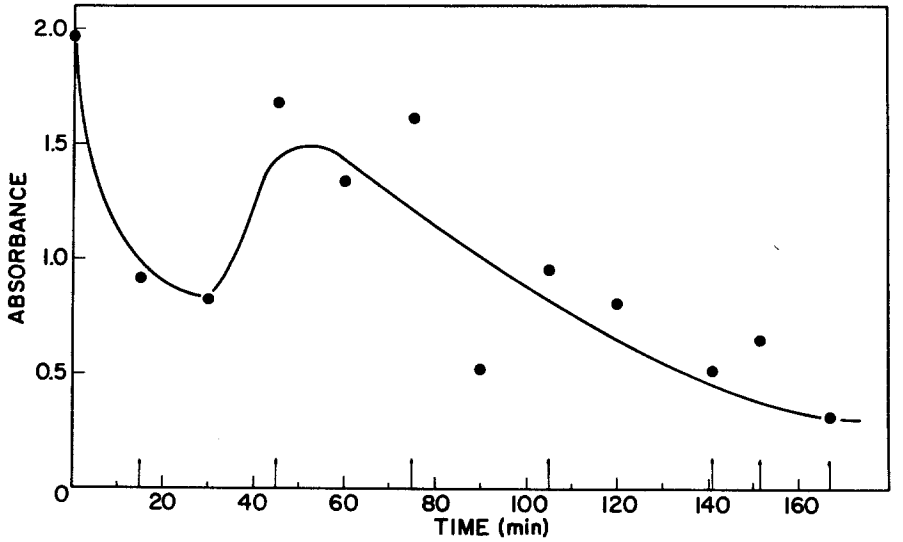

Figure 4. Reaction of phenylalanine with aqueous ClO_2 at 70° as monitored colorimetrically.

showed four spots (R_f 0.19, 0.38, 0.58 and 0.93). The spots at R_f 0.19, 0.38 and 0.58 were ninhydrin positive, but that at R_f 0.93 was ninhydrin negative. The residue from the reaction mixture could be separated into two fractions, an ether soluble fraction which contained the compounds associated with the spots at R_f 0.58 and 0.93, and a residual fraction, methanol soluble, which contained phenylalanine (R_f 0.38) and an unknown compound (R_f 0.19). The ether soluble compounds represented ca 5% of the original phenylalanine. Analysis by GC and GC-MS either directly or after formation of methyl esters identified these compounds as phenylacetic acid, benzoic acid, mandelic acid and phenylacetaldehyde (Table I), when compared to authentic standards. No phenylacetonitrile nor any chlorine containing compounds could be detected.

TABLE I

Compounds Identified From The Oxidation of Phenylalanine With Chlorine Dioxide

Name	Relative Retention Time[a]	Yield 70°C[b]	Yield 23°C[c]
Phenylalanine	-	65.6	85.7
Phenylacetic acid[d]	4.04	4.5	4.0
Phenylacetaldehyde	1.77	<0.1	trace
Benzoic acid[d]	2.11	0.4	0.1
Benzaldehyde	1.00	<0.1	trace
Mandelic acid[d]	9.53	0.2	0.1

[a] Retention times relative to benzaldehyde (1.03 mins.). [b] Reaction time 2 hours. [c] Reaction time 2 days. [d] Identified as methyl esters.

When the reaction was repeated at room temperature with a two molar equivalent of chlorine dioxide for 2 days, 86% of phenylalanine was unreacted and 4% yield of identified products were detected (Table I).

With a 10 molar excess of chlorine dioxide at room temperature for 6 days no phenylalanine remained neither could any of the products listed in Table I be detected by GLC or TLC nor was any ninhydrin positive material present.

The oxidation of phenylalanine by chlorine dioxide to give the products listed in Table I is consistent with the products obtained using other oxidizing agents. Phenylalanine has been reported to give benzoic acid and benzaldehyde with chromic acid (BLOCK and BOLLING 1945), phenylacetonitrile with Chloramine-T (MAHADEVAPPA and NAIDU 1976), and phenylacetonitrile and phenylacetaldehyde with aqueous chlorine (PEREIRA et al. 1973).

The extention of our work to the identification of reaction products under conditions used in water treatment plants needs

to be carried out.

ACKNOWLEDGEMENTS

KT thanks the Government of Canada for a visiting scientist fellowship.

REFERENCES

BARAKAT, M.Z., M.F. ABDEL-WAHAB and M.M. EL-SADR: J. Chem. Soc. 4685 (1956).

BENARDE, M.A., W.B. SNOW, V.P. OLIVIERI and B. DAVIDSON: Applied Microbiol. 15, 257 (1967).

BLOCK, R.J. and D. BOLLING: The Amino Acid Composition of Proteins and Foods, Charles C. Thomas, Springfield, Ill. U.S.A.; page 106, 1945.

BRENNER, M. and A. NIEDERWIESER: Experientia 16, 378 (1960).

CROLL, B.T.: Wat. Treat. Exam. Soc. Proc. 21, 213 (1972).

FRODYMA, M.M. and R.W. FREI: J. Chromatog. 15, 501 (1964).

FUJII, M. and M. UKITA: Nippon Nogei Kagaku Kaishi 31, 101 (1957).

GILBERT, E.: Ozone/Chlorine Dioxide Oxidation Products of Organic Materials. RICE, R.G. and J.A. CUTRUVO, Editors, Intl. Ozone Inst., Cleveland, Ohio 1978.

HODGEN, H.W. and R.S. INGOLS: Anal. Chem. 26, 1224 (1954).

KENNAUGH, J.: Nature 180, 238 (1957).

McCALDIN, D.J.: Chem. Rev. 60, 39 (1960).

MAHADEVAPPA, D.S. and H.M.K. NAIDU: Curr. Sci. 45, 652 (1976).

NORMAN, M.F.: Biochem. J. 30, 484 (1936).

PAVLOVA, L.B.: Chem. Abs. 55, 22464 (1961).

PALIN, A.T.: J. Inst. Wat. Engr. 28, 139 (1974).

PEREIRA, W.E., Y. HOYANO, R.E. SUMMONS, V.A. BACON and A.M. DUFFIELD: Biochim. Biophys. Acta 313, 170 (1973).

ROACH, D. and C.W. GEHRKE: J. Chromatog. 44, 269 (1964).

ROTH, M.: Anal. Chem. 43, 880 (1971).

SCHILLING, E.D., P.J. BURCHILL and R.A. CLAYTON: Anal. Biochem. 5, 1 (1963).

SCHMIDT, E. and F. BRAUNSDORF: Chem. Ber. 55, 1529 (1922).

TAYMAZ, K. and D.T. WILLIAMS: Int. J. Env. Anal. Chem. In Press (1979).

WELCHER, F.J.: Editor, Standard Methods of Chemical Analysis Sixth Edition. D. Van Nostrand Company, Inc. Princeton, New Jersey, Vol. 2, Part B, Page 2112 1966.

ZACHARIUS, R.M. and E.A. TAILLEY: J. Chromatog. 47, 51 (1962).