ELSEVIER

Journal of Chromatography A, 796 (1998) 283–288

JOURNAL OF CHROMATOGRAPHY A

Determination of hydroxyl radicals in an advanced oxidation process with salicylic acid trapping and liquid chromatography

Jen-Fon Jen[a,*], Meei-Fan Leu[a], Thomas C. Yang[b]

[a]*Department of Chemistry, National Chung-Hsing University, Taichung, 40227, Taiwan*
[b]*Department of Chemistry, National Kaohsiung Normal University, Kaohsiung, Taiwan*

Received 6 May 1997; received in revised form 7 July 1997; accepted 29 July 1997

Abstract

Liquid chromatography was used indirectly to detect hydroxyl radicals after a trapping reaction with salicylic acid in an advanced oxidation process. Through the quantitative determination of primary hydroxylated derivatives of salicylic acid, the concentration of hydroxyl radicals was evaluated relatively. Factors affecting the trapping reaction, such as the ratio of salicylic acid to hydrogen peroxide and trapping time, as well as the applicability of the method, were investigated. From the low relative standard deviation for replicate tests under controlled conditions, the method was found to be applicable to the determination of the generation rate or the concentrations of hydroxyl radicals in advanced oxidation processes for water treatment. © 1998 Elsevier Science B.V.

Keywords: Hydroxyl radicals; Salicylic acid

1. Introduction

During the past decade, there has been continuous interest in studies on mineralizing aqueous organic species with ''advanced oxidation processes'' [1–10]. In these processes, the hydroxyl radical, derived from the Fenton reaction [1,2], simple ozonation [3], ozone photolysis [4], semiconductor irradiation [5,6], photo-fenton reaction [7] and sonolysis [8–10], is employed as a powerful oxidant and a highly reactive transient that can rapidly oxidize most organic substances. However, the oxidation efficiency of hydroxyl radicals is usually limited by the rates of ·HO generation in solution. Therefore, increasing the ·HO generation rate and keeping it at a high level are the main factors that control the degradation efficiency of aqueous organic substances. Unfortunately, the degradation efficiency is still evaluated through the post-process determination of residuals, which is time-consuming and inefficient. Moreover, it is well known that the reaction pathways of radicals depend on the surroundings, and the efficiency of an advanced oxidation process can be affected greatly by the aqueous matrix. Therefore, an understanding of the generation rate and the concentrations of hydroxyl radicals in an advanced treatment process and then developing an appropriate strategy are required to obtain a high degradation efficiency.

Several methods have been developed to detect hydroxyl radicals, including EPR methods, which measure the electron paramagnetic resonance spectrum of a spin adduct derivative after spin trapping [11,12], or chromatographic methods, which deter-

*Corresponding author.

0021-9673/98/$19.00
PII S0021-9673(97)01019-4

mine the trapping products after they have been reacted with scavengers [13–15]. Because the EPR methods require a sophisticated high-cost instrument system and a skillful operator, they are not suitable for routine analysis.

High-performance liquid chromatography (HPLC) has been employed to measure hydroxyl radicals in vivo, by determining the hydroxylated derivatives of salicylic acid [15]. Generally, the concentration of hydroxyl radicals in in-vivo samples is very low. The trapping reaction was achieved at physiological pH (pH 7.4). However, in an advanced oxidation, hydroxyl radicals are produced in relatively large amounts, and the system is usually optimized for a specific pH range, such as pH 3–4 for Fenton's oxidation [1,2,16]. It is well known that radical reactions are matrix-dependent and that the formation of derivatives of salicylate in the trapping reaction is also pH-dependent. Therefore, the use of the HPLC method, including trapping with salicylic acid for hydroxyl radical determination in an advanced oxidation process, is worthy of examination.

The purpose of this study was to examine the appropriateness of the HPLC method for determining the concentration of hydroxyl radicals in advanced oxidation processes. The hydroxyl radicals were generated by Fenton's reaction, and then trapped by salicylic acid. A conventional electrochemical detector coupled with a UV detector was used to monitor the hydroxylated derivatives and salicylic acid after they had been separated using HPLC. From the total concentration of hydroxylated derivatives, the hydroxyl radicals used for hydroxylation can be obtained, and the formation of hydroxyl radicals under the specified conditions can be estimated relatively.

2. Experimental

2.1. Apparatus

An LC 1100 pump module (ICI Instruments, Australia) equipped with a Rheodyne 7125 injection valve (Cotati, CA, USA) and a Supelcosil LC-8 reversed-phase column (250×4.6 mm I.D., 5 μm) (Supelco, Bellefonte, PA, USA) were used as the chromatographic system. An LC 1260 EC detector (ICI Instruments), set at 0.80 V (vs. Ag/AgCl), in conjunction with a DP 800 analysis system (ICI Instruments), was used to detect and collect chromatographic data. A Waters 484 tunable UV–VIS detector (Waters, Milford, MA, USA) was also used to identify the separated species at 296 nm. The eluent flow-rate was programmed as follows: 1.0 ml/min for the first 14 min, then at 1.5 ml/min for 10 min, and then back to 1.0 ml/min for 4 min before the next injection.

2.2. Chemicals and reagents

Deionized water was produced using a Barnstead Nanopure water system (Thermolyne, Dubuque, IA, USA) and was used for all aqueous solutions. All chemicals were of ACS reagent grade. Standard stock solutions (1000 μg/ml) of salicylic acid, 2,3-dihydroxybenzoic acid (2,3-dHBA), 2,5-dihydroxybenzoic acid (2,5-dHBA) and catechol (Riedel-de Haën, Hannover, Germany) were prepared by dissolving 0.100 g of them in 50 ml of water and then adding water to adjust the volume to 100 ml. A 1000 μg/ml ferrous solution was prepared by dissolving 0.702 g of $(NH_4)_2[Fe(SO_4)_2]\cdot 6H_2O$ (Riedel-de Haën) in 100 ml water, and 3% H_2O_2 was prepared by diluting 10 ml of 30% H_2O_2 (Baker, Phillipsburg, WI, USA) to 100 ml with water. All of the solutions were stored in brown glass bottles, and kept in a 4°C refrigerator for a maximum of two weeks. Fresh working solutions were prepared daily by appropriate dilution of the stock solutions. The HPLC eluent was prepared using 10% (v/v) HPLC-grade acetonitrile, 10% methanol (Mallinckrodt, Paris, KY, USA), 0.03 *M* citric acid (Baker), 0.3 *M* acetic acid (Riedel-de Haën) and water. Sulfuric acid (0.01 *M*) was used to adjust the pH. All eluents were filtered through a 0.45 μm PVDF membrane filter and degassed ultrasonically.

2.3. Generation and trapping of OH radicals

The generation and trapping of OH radicals was achieved by introducing 10 ml of a 100 μg/ml H_2O_2 solution (pH 3.0) into 25 ml reaction vessels, mixing it with 10 ml volumes of solutions containing appropriate amounts of salicylic acid and 10 μg/ml of ferrous ion. Samples were then taken for HPLC analysis at various times throughout the reaction

process. Reactions were maintained at 25°C using a water bath.

3. Results and discussion

3.1. Separation and identification of species

In in-vivo studies, only 2,3-dHBA and 2,5-dHBA were found as the hydroxylated derivatives of salicylic acid in hydroxyl radical trapping. Catechol was often ignored due to its insignificant distribution under the conditions used [15]. However, in our study, a rather high yield of catechol was found in lower pH acidic trappings. Hence, separation reactions involved 2,3-dHBA, 2,5-dHBA, catechol and salicylic acid. The separation was achieved with a reversed-phase C_8 column under the elution conditions described previously. Because of the oxidation inactivity of salicylic acid under the 0.80 V (vs. Ag/AgCl) detection potential of the EC detector, a UV detector was coupled to the system to monitor salicylic acid at 296 nm. Because the reactive character of the EC detector may affect the analytes, the UV detector was linked in front of the EC detector. Chromatograms of the hydroxylated derivatives in an authentic sample are shown in Fig. 1. To identify these species, not only were the retention behaviors compared with the standards, but they were also reconfirmed using a UV–Vis spectrophotometer, following fraction collection. As shown in the chromatograms, four species are well separated within 23 min. Peaks 1 to 4 agreed well with 2,5-dHBA, catechol, 2,3-dHBA and salicylic acid, respectively.

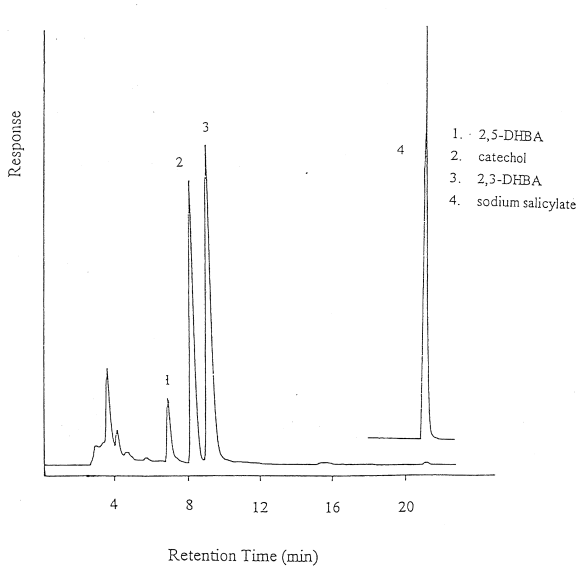

Fig. 1. Chromatograms of the hydroxylated derivatives and salicylic acid. Peaks 1 to 4 are 2,5-dHBA, catechol, 2,3-dHBA and salicylic acid, respectively.

3.2. Calibration plots and reproducibilities for the hydroxylated derivatives

In order to test the applicability of the HPLC method for the quantitative determination of hydroxylated derivatives and salicylic acid, calibration plots were determined for these four species over the concentration ranges summarized in Table 1. The linear relationship between the peak area and the injected quantity was very good for the four species. The correlation coefficients were all above 0.999 for the following concentration ranges: 0.05 to 26 μg/ml of 2,5-dHBA, 0.05 to 20 μg/ml of catechol, 0.05 to 40 μg/ml of 2,3-dHBA and 1.0 to 600 μg/ml of salicylic acid. The repeatability was examined by injecting 20-μl volumes of the lowest and highest concentrations of each species, determined from the calibration plots, in triplicate. The R.S.D.s of retention times and peak areas were all within 0.8 and 3.0%, respectively. The detection limits were calculated from three times the average background noise, and were 0.1 ng for 2,5-dHBA, catechol and 2,3-dHBA, and 1.0 ng for salicylic acid.

3.3. Quantitative effect of salicylic acid in radical trapping

The effective trapping of hydroxyl radicals by salicylic acid, which produces the primary derivatives 2,5-dHBA, catechol and 2,3-dHBA, can be used for quantitative determination. However, a competition equilibrium is established between the salicylic acid and the primary derivatives for trapping the hydroxyl radicals. Table 2 lists the relative rate constants for the hydroxylation of salicylic acid and its primary derivatives. It can be seen that although only the 2,5-dHBA has a higher rate constant than salicylic acid, the whole primary

Table 1
Detection characteristics of hydroxylated derivatives

Analyte	Linear dynamic range (μg/ml)	Regression equation	Correlation coefficient	Detection Limit (ng)
2,5-dHBA	0.05–26	$Y=569138X+24779$	0.9999	0.1
Catechol	0.05–20	$Y=829841X+140267$	0.9998	0.1
2,3-dHBA	0.05–40	$Y=608506X-85744$	0.9999	0.1
Salicylic acid	1.0–600	$Y=16457X-12585$	0.9999	1.0

[a]Based on a signal-to-noise ratio of three ($S/N=3$).

Table 2
Rate constants for the hydroxylation of salicylic acid and its primary derivatives

Species	Rate constant, k (l mol^{-1} s^{-1})	Relative rate constant, K_{rel}	Reference
2,5-dHBA	$k_1=2.4\times10^{10}$	$K_{rel}=k_1/k_4=1.09$	[14]
Catechol	$k_2=1.1\times10^{10}$	$K_{rel}=k_2/k_4=0.50$	[17]
2,3-dHBA	$k_3=1.3\times10^{10}$	$K_{rel}=k_3/k_4=0.59$	[14]
Salicylic acid	$k_4=2.2\times10^{10}$	$K_{rel}=k_4/k_4=1.00$	[14]
$k_{sum}=k_1+k_2+k_3=4.8\times10^{10}$		$K_{rel}=(k_1+k_2+k_3)/k_4=2.18$	

derivatives are in priority to salicylic acid in hydroxyl radical trapping. Therefore, an excess of salicylic acid is required to ensure that the trapping is effective. Fig. 2 shows the effectiveness of trapping for various concentrations of salicylic acid. As predicted, the effective trapping increases with increasing amounts of salicylic acid in the low concentration range (up to 250 μg/ml) and then levels off. As the hydroxyl radicals are generated from the decomposition of hydrogen peroxide, catalyzed by ferrous ion, the ratio of salicylic acid to hydrogen peroxide should be considered. In Fig. 2, it is obvious that the effective trapping becomes stable at a ratio of 5:1. Considering the capacity of the separation column, 250 μg/ml salicylic acid (ratio 5:1) was used to trap the hydroxyl radicals throughout the studies.

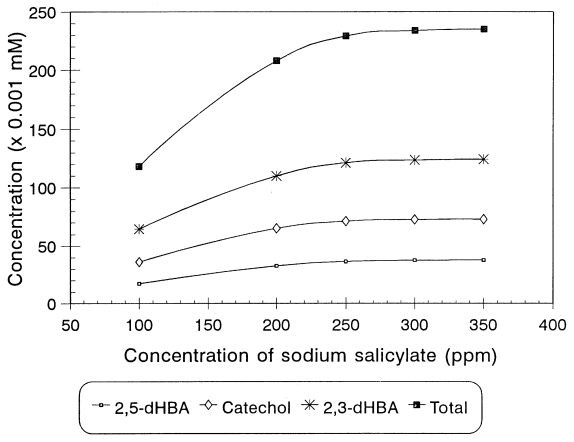

Fig. 2. The effectiveness of trapping for various concentrations of salicylic acid. Hydroxyl radicals were generated using 50 μg/ml H_2O_2 and 5 μg/ml of a ferrous solution at pH 3.0, and trapped by salicylic acid for 35 min.

3.4. Time required to trap the primary hydroxylated derivatives

An ideal analytical technique should be able to complete sample preparation and analysis within a short time. The reactions between hydroxyl radicals and salicylic acid, as well as its primary hydroxylated derivatives, are very fast, and have the rate constants listed in Table 2. Although an excess of salicylic acid was added, the time required to obtain sufficient amounts of primary derivatives for quantitative determination should be investigated. Fig. 3 demonstrates the net formation of primary derivatives for various reaction times. It can be seen that the net formation of primary derivatives increased smoothly with time. It indicates that the generation and trapping of effective hydroxyl radicals for quantitative determination are under control in the studied conditions within a 35-min trapping reaction. In our studies, a 15-min trapping reaction was selected.

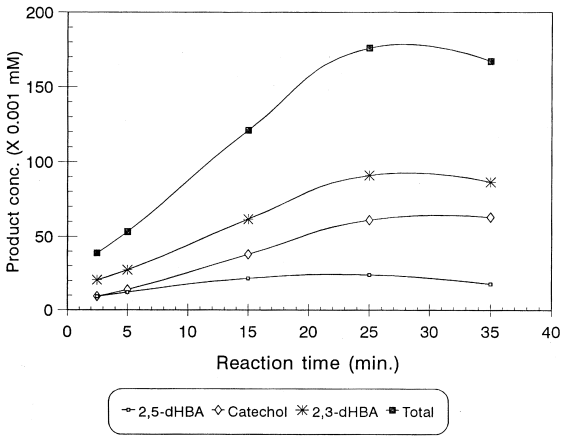

Fig. 3. The net formation of primary derivatives at various reaction times. Hydroxyl radicals were generated as described in Fig. 2, and trapped using 250 μg/ml of salicylic acid.

3.5. Reproducibility of the hydroxyl radical trapping process

In order to test the applicability of the method for the quantitative determination of primary hydroxylated derivatives, for evaluation of the generation rate or the concentrations of hydroxyl radicals, a solution containing 5 μg/ml of Fe^{2+} and 50 μg/ml of H_2O_2, pH 3.0, was used to generate hydroxyl radicals, and 250 μg/ml of salicylic acid were used to trap the radicals for 15 min. The primary hydroxylated derivatives of salicylic acid (2,5-dHBA, catechol and 2,3-dHBA) were then separated and determined using the HPLC system. The same experiment was repeated five times. The production of 2,5-dHBA, catechol and 2,3-dHBA was very reproducible, with R.S.D.s of 3.2, 2.0 and 3.8%, respectively. By evaluating the effective hydroxyl radical trappings from the summation of these products, the R.S.D. was found to be 3.1%, which is acceptable.

3.6. Determination of hydroxyl radicals generated at various pH values

In order to investigate the generation of hydroxyl radicals by Fenton's reaction at various pH values, reactions were carried out in water that had been adjusted to pH values of 2.0, 2.5, 3.0, 3.5, 4.0, 4.5 and 5.0 with sulfuric acid. After being trapped by salicylic acid, the generation of hydroxyl radicals was evaluated through the determination of hydroxylated derivatives by HPLC. Table 3 lists the yields of hydroxylated derivatives and their summation after a 15-min trapping reaction. It can be seen that the generation of hydroxyl radicals (sum of products) increased with increasing pH, was optimal at pH 3.5 and thereafter decreased. This matches the pH for the optimal degradation efficiency of organic species by Fenton's oxidation.

4. Conclusion

In this study, HPLC, with radical trapping by salicylic acid, has proven to be an applicable method for indirectly determining the relative concentrations of hydroxyl radicals in aqueous solution under

Table 3
Yields of hydroxylated derivatives and hydroxyl radicals generated by Fenton's reaction at various pH values

pH	2,5-dHBA (μ*M*)	Catechol (μ*M*)	2,3-dHBA (μ*M*)	Hydroxyl radicals (μ*M*)[b]
2.0	1.98 (0.7%)[a]	29.68 (2.7%)	18.44 (2.6%)	50.10 (3.8%)
2.5	6.63 (1.9%)	43.11 (2.1%)	37.49 (2.6%)	87.23 (3.8%)
3.0	20.53 (2.5%)	28.28 (2.0%)	49.31 (2.9%)	98.12 (4.3%)
3.5	27.61 (1.5%)	26.26 (3.1%)	57.78 (3.4%)	11.165 (4.8%)
4.0	21.97 (0.5%)	9.48 (0.9%)	30.68 (2.6%)	62.13 (2.8%)
4.5	15.55 (2.0%)	4.15 (3.3%)	19.27 (2.8%)	38.97 (4.8%)
5.0	7.64 (1.9%)	1.38 (1.2%)	11.10 (3.4%)	20.12 (4.1%)

Hydroxyl radicals were generated using 50 μg/ml H_2O_2 and 5 μg/ml of a ferrous solution, and trapped using 250 μg/ml of salicylic acid for 15 min.

[a]Average data of three determinations, the data in parentheses are relative standard deviations.

[b]The hydroxyl radicals were evaluated by the sum of hydroxylated derivatives.

controlled trapping conditions. The ratio of salicylic acid to hydrogen peroxide should be over 5.0, for a trapping time of about 15 min. The concentrations of hydroxyl radicals in an advanced oxidation processes can thus be determined using this method.

Acknowledgements

The authors thank the National Science Council of the R.O.C. for financial support under grant number NSC 85-2113-M-005-012.

References

[1] J.J. Pignatello, Environ. Sci. Technol. 26 (1992) 944–951.
[2] J.B. Carberry, S.H. Lee, ACS Symp. Series 544 (1994) 197–222.
[3] J. Hoigne, H. Bader, Water Res. 10 (1976) 377–386.
[4] G.R. Peyton, W.H. Glaze, Environ. Sci. Technol. 22 (1988) 761–767.
[5] M.R. Prairie, L.R. Evans, B.M. Stange, S.L. Marthinez, Environ. Sci. Technol. 27 (1993) 1776–1782.
[6] E. Pramauro, M. Vincenti, Environ. Sci. Technol. 27 (1993) 1790–1795.
[7] J.J. Pignatello, G. Chapa, Environ. Sci. Technol. 13 (1994) 423–427.
[8] G.E. Orzechowska, E.J. Poziomek, V.F. Hodge, W.H. Engelmann, Environ. Sci. Technol. 29 (1995) 1373–1379.
[9] A. Bhatnagar, H.M. Cheung, Environ. Sci. Technol. 28 (1994) 1481–1486.
[10] H.M. Cheung, S. Kurup, Environ. Sci. Technol. 28 (1994) 1619–1622.
[11] V. Misik, N. Miyoshi, P. Riesz, J. Phys. Chem. 99 (1995) 3605–3611.
[12] V. Misik, L.J. Kirschenbaum, P. Riesz, J. Phys. Chem. 99 (1995) 5970–5976.
[13] S. Fukui, Y. Hanasaki, S. Ogawa, J. Chromatogr. 630 (1993) 187–193.
[14] M.A. Oturna, J. Pinson, J. Phys. Chem. 99 (1995) 13948–13954.
[15] M. Grootveld, B. Halliwell, J. Biochem. 237 (1986) 499–504.
[16] D.L. Sedlak, A.W. Andren, Environ. Sci. Technol. 25 (1991) 1419–1427.
[17] G.V. Boxton, C.L. Greenstock, W.P. Hilman, A.B. Ross, J. Phys. Chem. Ref. Data 17(2) (1988) 706.