Available online at www.sciencedirect.com
ScienceDirect
Journal of Environmental Sciences 20(2008) 28–32
JOURNAL OF ENVIRONMENTAL SCIENCES
ISSN 1001-0742
CN 11-2629/X
www.jesc.ac.cn

Determination of atmospheric hydroxyl radical by HPLC coupled with electrochemical detection

LIU Bin, WANG Hui-xiang*

College of Environmental Sciences, Peking University, Beijing 100871, China. E-mail: ysliubing@gmail.com

Received 5 March 2007; revised 18 April 2007; accepted 24 April 2007

Abstract

The hydroxyl radical (·OH) plays a central role in the oxidation and removal of many atmospheric compounds. Measurement of atmospheric ·OH is very difficult because of its high reactivity and low atmospheric abundance. In this article, a simple and highly sensitive method, high performance liquid chromatography coupled with coulometric detection (HPLC-CD), was developed to determine ·OH indirectly by determining its reaction products with salicylic acid (SAL), 2,3-dihydroxybenzoic acid (2,3-DHBA), and 2,5-dihydroxybenzoic acid (2,5-DHBA). Under the optimum conditions for its determination, 2,3-DHBA and 2,5-DHBA could be well separated and the detection limits for 2,3-DHBA were 3×10^{-10} mol/L and for 2,5-DHBA were 1.5×10^{-10} mol/L, which were lower than most previous reports. This method was also applied to measure atmospheric hydroxyl radical levels and demonstrated the feasibility in clean and polluted air.

Key words: electrochemical detection; HPLC; hydroxyl radical; troposphere

Introduction

The hydroxyl radical (·OH) is the main constituent of the oxidizing potential of the troposphere. It causes the transformation of many trace components into water-soluble forms, which can then be removed from the troposphere. ·OH has therefore acquired the title "detergent of the atmosphere" and the level of ·OH reflects the earth's "oxidizing capacity" (Thompson, 1992). It is confirmed that the behavior of the hydroxyl radical has an important influence on the pollution in the region and even on global environmental issues, such as, acid rain, the greenhouse effect, and so on. Because of its high reactivity, the hydroxyl radical has a very short lifetime and is therefore present in extremely low concentrations. The mid-day range typically varies from about 10^6 to 10^7 radicals/cm^3 (Eisele and Bradshaw, 1993; Kramp and Volz-Thomas, 1997).

Ever since the importance of ·OH radicals in the troposphere had been identified, the measurement techniques have long been a goal, to probe short-term variations of tropospheric hydroxyl radicals, especially since the 1990s. The first ·OH measurement was reported by Wang and Davis in 1974, and there have been a number of reviews that summarize techniques used to detect atmospheric ·OH (Heard and Pilling, 2003). At present several ·OH measurements have achieved considerable success, such as, laser-induced fluorescence (Ren *et al*., 2005; Heard, 2006), differential optical absorption spectroscopy (Brauers *et al*., 2001), chem-ionization mass spectrometry (Berresheim *et al*., 2002), radiocarbon method (Campbell *et al*., 1995), electron spin resonance (ESR) (Ma *et al.*, 1999), and salicylic acid method (Salmon *et al*., 2004).

Previous studies have shown that salicylic acid (SAL) reacts with ·OH with high specificity and provides a fast, reproducible and well-characterized response (Fig.1). The derivatives (2,3-dihydroxybenzoic acid (2,3-DHBA) and 2,5-dihydroxybenzoic acid (2,5-DHBA)) can be detected with high sensitivity and selectivity. The hydroxylated products generated from the reaction are separated and measured by high performance liquid chromatography (HPLC) coupled with ultraviolet (UV), fluorescence detection or mass spectrometry (MS). Application of this method, to measure atmospheric gas phase ·OH, has been studied at Washington State University (Chen and Mopper, 2000), the Max-Planck-Society, Germany (Cooke and Oberlander, 1999), York University, Toronto (Salmon *et al*., 2004), and Peking University (Ren *et al*., 2002). In their studies, fluorescence detection has been chosen to determine the derivatives and the detection limit was $(3–10) \times 10^5$ radicals/cm^3 with sampling integration of about 1 h. Although the needed averaging time is probably long compared to spectroscopic measurement techniques, the major attributes of this method are its compactness, portableness, and inexpensiveness.

Other than the fluorescence detection, Floyd *et al*. (1984) developed a sensitive electrochemical detector for the detection of hydroxyl-free radical generation in various systems. Subsequently, this method was improved

*Corresponding author. E-mail: hxwang@plcu.edu.cn.

jesc.ac.cn

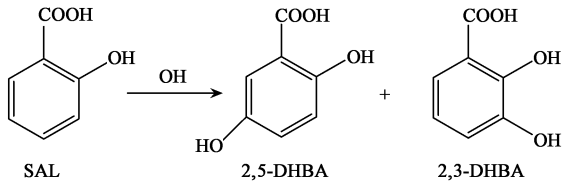

Fig. 1 Reaction of salicylic acid (SAL) with ·OH.

and became the most important method for the *in vivo* measurement of the hydroxyl radical in the biological and clinical areas (McCabe *et al*. 1997; Blandini *et al*, 1999). The electrochemical method had the advantages of being simple, sensitive, portable, and economic. The sensitivity of electrochemical detection was reported to be about 1000 times higher than ESR or HPLC with UV-Vis detection (Kilnic, 2005). In this article, this new method was transferred from the biochemical to atmospheric analysis, to detect atmospheric gas phase ·OH. Two electrochemical detection methods, coulometric detection and amperometric detection, were established, and the optimum conditions were explored. The more sensitive one, based on coulometric detection, was employed in the measurement of the atmospheric hydroxyl radical concentrations of Beijing. An intercomparison test between HPLC coupled with electrochemical detection and fluorescence detection was carried out to compare the two different sets on the same ·OH samples.

1 Establishment of electrochemical detections

1.1 Chemicals and reagents

HPLC-grade methanol, isopropanol, and acetonitrile were obtained from Fisher (Beijing, China). 2,3- and 2,5-dihydroxybenzoic acids (DHBAs) were purchased from Sigma (St. Louis, MO, USA). SAL (99.5%), citric acid, sodium acetate, sodium citrate, sodium dodecyl sulfate, and ethylenediamine tetraacetic acid (EDTA) were purchased from J&K (Beijing, China). HPLC mobile phases were prepared from HPLC-grade H_2O. Membranes (0.45 μm pore size) were used for filtration of the samples.

1.2 HPLC measurement

The liquid chromatographic system (Agilent 1100 series) was equipped with a pump, a valve injector, an online degasser, and chromatographic data processing software (HP ChemStation for LC). The separation was performed using a C_{18} reversed-phase column (5 m, 250 mm × 4.6 mm). A coulometric detector (CouloChem III Coulomb array detector, ESA, USA) and an amperometric detector (Bioanalytical System, West Lafayette, IN, USA) were used to probe the hydroxylated derivatives. The coulometric detector was equipped with a dual electrode analytical cell (Coulochem 5010, ESA) and a protection electrode (Coulochem 5020, ESA). Model 5010 cell was comprised of two flow-through porous graphite coulometric electrodes. The amperometric detection was equipped with a glassy carbon working electrode, a saturated calomel reference electrode, and a platinum wire counter electrode.

1.3 Conditions of amperometric detection

The mobile phase was comprised of 50 mmol/L sodium acetate, 10 mmol/L citric acid, 0.15 mmol/L EDTA, 0.43 mmol/L sodium dodecyl sulfate, 5% acetonitrile, and was adjusted to pH 3.4. EDTA was added to restrict the influence of the metal ions and other impurities. Sodium dodecyl sulfate was used to enhance the signal of the derivatives. Elution was performed at a flow-rate of 0.8 ml/min and at a column temperature of 25°C. Prior to use, the surface of the carbon electrode was polished with emery paper and alumina powder respectively, and then sonicated in doubly distilled water for 3 min to clean it thoroughly.

As there are electroactive hydroxyl groups in their molecular structures, 2,3-DHBA and 2,5-DHBA molecules can be oxidized at a carbon electrode and produce current responses. Fig.2 shows the hydrodynamic voltammograms of these analytes, which are obtained by monitoring their current responses after HPLC separations at the applied potential range from 500 to 1000 mV. It was found that the current responses of these analytes increased with the enhancement of the applied potential. For determining 2,3-DHBA and 2,5-DHBA together and their best signal-to-noise ratio, 800 mV (vs. Ag/AgCl) was selected as the detection potential in this experiment.

1.4 Conditions of coulometric detection

The mobile phase contained 50 mmol/L sodium acetate, 50 mmol/L sodium citrate, 8% (v/v) methanol, and 2% (v/v) isopropanol and was adjusted to pH 2.75. Elution was also performed at a flow-rate of 0.8 ml/min and at a column temperature of 25°C. Analytes were detected on a dual electrode analytical cell with the first electrode set to oxidize DHBAs and the second electrode set to oxidize SAL. A guard cell was placed between the pump and the auto sampler with a potential of 800 mV (vs. Pd), to oxidize contaminants in the mobile phase. The correct choice of applied potentials to the two electrodes was obtained from the current-voltage (CV) curves of the analytes. CV curves (Fig.3) were generated by analyzing the same concentration of 2,3-DHBA, 2,5-DHBA, and

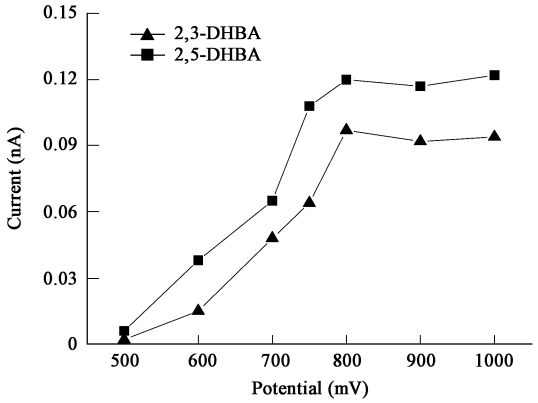

Fig. 2 Hydrodynamic voltammograms of 2, 3-DHBA and 2, 5-DHBA, with the same concentration of 3×10^{-8} mol/L by amperometric detection, under different detection voltages. Other conditions were similar to those for optimum conditions.

jesc.ac.cn

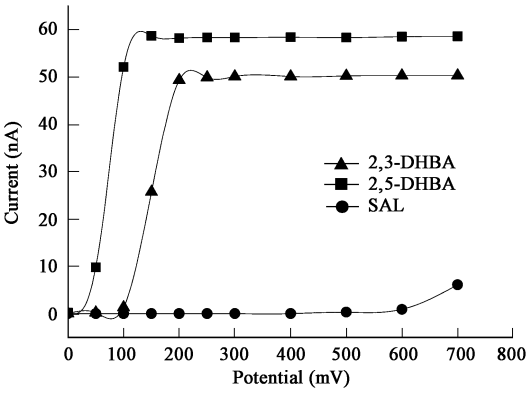

Fig. 3 Hydrodynamic voltammograms of 2,3-DHBA, 2,5-DHBA, and SAL, with the same concentration of 3×10^{-8} mol/L by coulometric detection, under different detection voltages. Other conditions were similar to those for optimum conditions.

SAL with 3×10^{-8} mol/L. From these data the major differences in maximal oxidation potentials allowed the system to be configured so that the first electrode measured DHBAs (+ 250 mV vs. Pd) and the second measured SAL (+ 750 mV vs. Pd).

1.5 Linearity, repeatability, and detection limits

A series of standard solutions of 2,3-DHBA and 2,5-DHBA with a concentration in the range of 1.0×10^{-7} to 1.0×10^{-10} mol/L were analyzed under optimal conditions by the two detectors. The results are shown in Table 1. The earlier results showed that coulometric detection was superior to amperometric detection in the determination of DHBAs. For 2,5-DHBA, the detection limit of coulometric detection was 0.15 nmol/L, which was lower than most previous reports (Jen and leu, 1998; Ren *et al*., 2001b). Although Salmon (Salmon *et al*., 2004) achieved a 47 pmol/L detection limit with fluorescence detection, this more simple and convenient method was sensitive enough for the measurement of ·OH in clean and polluted air.

2 Ambient measurements and intercomparison tests

2.1 Air sampling

The sampling site was located in Beijing, China, and the air sampler was placed outside the second floor of the old geosciences building on the campus of Peking University, Beijing, about 100 m away from a major traffic street. ·OH was quantitatively scrubbed from the air with a wet denuder sampler. SAL, with the concentration of 50 μmol/L, was injected into the denuder with the help of a peristaltic pump and the SAL membrane was formed in the rolling denuder under the function of a motor. The air sample was pumped into the denuder with a flow rate of 30 L/min and the length of denuder ensured the retention time was enough for the process of ·OH diffusion. More details of the wet denuder sampler were described in Ren *et al*. (2001a).

KI of 10 μmol/L, was added into the solution as it was known to remove O_3, NO_2, H_2O_2, O_2 ($^1\Delta$), and other free radicals. Salmon (Salmon *et al*., 2004), suggested that approximately 10 μmol/L KI was sufficient to remove up to 80 ppbv ozone in 42 μmol/L SAL. As some of the interferential reactions were pH-dependent, such as, HO_2, O_2 ($^1\Delta$), these pH-related interferences could be suppressed by sampling, under acidic conditions. In this experiment the pH was adjusted to 4.6. Although pollutants, such as, O_3, NO_2, H_2O_2, CH_3O_2, and PAN, might cause elevated or depressed concentration of ·OH, concentrations of these pollutants were not monitored in this preliminary study.

2.2 Quantification and assumption

It is assumed that all SAL loss is because of the reaction of ·OH: $C_{\mathrm{SALr}} = C_{\cdot\mathrm{OH}}$. The fraction of 2,5-DHBA can be defined as:

$$F_{2,5\text{-DHBA}} = C_{2,5\text{-DHBA}}/C_{\mathrm{SALr}} = C_{2,5\text{-DHBA}}/C_{\cdot\mathrm{OH}} \quad (1)$$

Thus, when $F_{2,5\text{-DHBA}}$ is given, $C_{\cdot\mathrm{OH}}$ can be estimated by the detection of the concentration of 2,5-DHBA formed during air sampling. If the loss of ·OH in the inlet surface and during transport from gas phase to gas-liquid interface can be neglected, $F_{2,5\text{-DHBA}}$ obtained from the liquid phase can be used to quantify the atmospheric ·OH concentration. Here, the concentrations of atmospheric ·OH have been calculated by using Eq. (2) in which V_{f} is the post-reaction volume of SAL, F_{g} is the air flow rate, t is the sampling time, T_0 and T are the standard and local temperatures and P_0 and P are the standard and local atmospheric pressures.

$$C_{\mathrm{OH}} = \frac{C_{2,5\text{-DHBA}} P T_0 V_f N_A}{F_{2,5\text{-DNPH}} P_0 T F_{\mathrm{g}} t} \quad (2)$$

2.3 Intercomparison results

An intercomparison was designed to evaluate the accuracy and agreement between the two detections. Ambient samples outside the laboratory were collected from 11:00 to 14:00 local time during April 9–15. Samples collected were separated by the HPLC system and determined by the following fluorescence and coulometric detection. The

Table 1 Regression equation and detection limit

	Amperometric detection		Coulometric detection	
	2,3-DHBA	2,5-DHBA	2,3-DHBA	2,5-DHBA
Regression equation	$1.18\times10^8\times C - 0.10$	$1.22\times10^8\times C + 0.28$	$1.50\times10^9 C + 1.14$	$1.75\times10^9\times C + 1.83$
Detection limit (nmol/L)	3	2	0.3	0.15
r^2	0.9946	0.9924	0.9983	0.9969
RSD	3.25%	3.79%	1.87%	2.91%

Detection limit was estimated according to thrice the signal-to-noise ratio; in the regression equation, C (mol/L) is the concentration of 2,3-DHBA or 2,5-DHBA.

jesc.ac.cn

mobile phase and the conditions of coulometric detection have been presented in 2.4. The excitation and emission wavelengths of the fluorescence detection were set at 314 and 438 nm, respectively. The comparison of the data obtained by the two detections is shown in Fig.4. The ·OH concentrations obtained agreed well with the linear correlation coefficient square (r^2 = 0.9983, n = 26). Variations of 75% data were under 10% and only three of them were up to 20%–25%. These results demonstrated that accurate measurements could be conducted by electrochemical detection.

2.4 Ambient measurement

The measurement system was employed in the atmospheric measurement during 16–18 April, 2005. Samples of 15-min were collected every hour to denote the hour average. As only to demonstrate the feasibility of this method, concentrations of other pollutants were not monitored in this preliminary study. As shown in Fig.5, the derivatives (2,3-DHBA and 2,5-DHBA) were well separated in 30 min and no interferential substance was observed. The variations of ·OH concentrations during the measurement period are shown in Fig.6. The concentrations of ·OH during the period were in the range of (3.0–14.2) × 10^6 radicals/cm^3. This range was similar to that reported by Ren *et al*. (2002), at the same site. The peak of ·OH concentrations appeared from 12:00 to 14:00 and during the night ·OH concentrations were always lower, even less than 20% of the sunny mid-day ·OH concentrations.

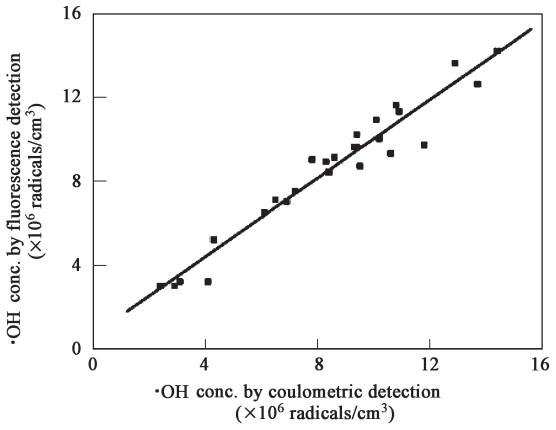

Fig. 4 Intercomparison of ·OH concentration detected by fluorescence and coulometric during 9–15 April 2005.

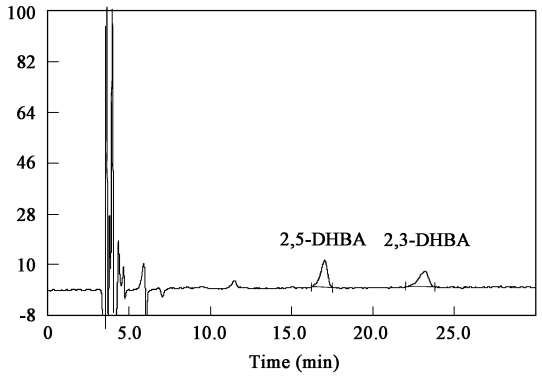

Fig. 5 Chromatogram of an air sample obtained by coulometric detection.

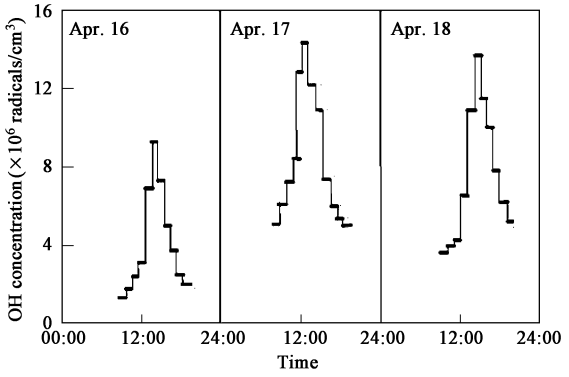

Fig. 6 Atmospheric ·OH concentration variations from 16–18 April, 2005. Samples of 15-min were collected every hour, which denotes the hour average.

3 Conclusions

Two electrochemical detections were employed to evaluate the determination of the tropospheric hydroxyl radical by liquid phase scrubbing. The coulometric detection achieved a well-pleasing detection limit and was applied to detect the gas phase ·OH together with fluorescence detection. An intercomparison test showed that the two methods agreed well when applied to monitor ambient ·OH concentrations. The detection limit of the gas phase ·OH was 9.2×10^5 radicals/cm^3 in the air, which was similar to those reported in literature. Although this method is still under development, the preliminary results presented in this communication indicate the feasibility of this approach for the measurement of ·OH in clean and moderately polluted air.

To validate the results of this study and to facilitate the field application of this method, a study is underway to build a calibrated gas phase ·OH standard source, to validate the linear response to gas phase ·OH, test possible interferences by controlled experiments using known amounts of potential interfering species, improve the sampling time resolution, and to automate the system by directly coupling the sampling with the chromatography.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 40075026). The authors thank Sihua Lu and Huan Wang for their laboratory assistance.

References

Berresheim H, Elste T, Tremmel H G, Allen A G, Hansson H C, Rosman K *et al*., 2002. Gas-aerosol relationships of H_2SO_4, MSA, and ·OH: Observations in the coastal marine boundary layer at Mace Head, Ireland. *J Geophys Res-Atmos*, 107: 8100–8107.

Blandin F, Martignoni E, Ricotti R, 1999. Determination of hydroxyl free radical formation in human platelets using

jesc.ac.cn

high-performance liquid chromatography with electrochemical detection. *J Chromatogr B*, 732: 213–220.

Brauers T, Hausmann M, Bister A, Kraus A, Dorn H, 2001. ·OH radicals in the boundary layer of the Atlantic Ocean: 1. Measurements by long-path laser absorption spectroscopy. *J Geophys Res-Atmos*, 106: 7399–7414.

Campbell M J, Hall B D, Sheppard J C, Utley P L, O'Brien R J, Hard T M, George L A, 1995, Intercomparison of local hydroxyl measurements by radiocarbon and FAGE techniques. *J Atmos Sci*, 52: 3421–3427.

Chen X, Mopper K, 2000. Determination of tropospheric hydroxyl radical by liquid phase scrubbing and HPLC: Preliminary results. *J Atmos Chem*, 36: 81–105.

Cooke K M, Oberlander E A, 1999. Development of a technique to measure tropospheric ·OH using liquid derivatisation and HPLC with fluorescence detection. In: Proceedings of the Eurotrac-2 Symposium'98 on "Transport and Chemical Transformation of Pollutants in the Troposphere" Garmisch-Partenkirchen 1998, 1 (P. M. Borrell, Borrell P., eds.). Southampton, England: WIT Press. 375–379.

Eisele F L, Bradshaw J D, 1993. The elusive hydroxyl radical: measuring ·OH in the atmosphere. *Anal Chem*, 65: 927–939.

Floyd R A, Watson J J, Wong P K, 1984. Sensitive assay of hydroxyl free radical formation utilizing high pressure liquid chromatography with electrochemical detection of phenol and salicylate hydroxylation products. *J Biochem Biophys Methods*, 10: 221–235.

Heard D E, Pilling M J, 2003. Measurements of ·OH and HO_2 in troposphere. *Chem Rev*, 103: 5163–5198.

Heard D E, 2006. Atmospheric field measurements of the hydroxyl radical using laser-induced fluorescence spectroscopy. *Annual Review of Physical Chemistry*, 57(1): 191–216.

Jen J F, Leu M F, 1998. Determination of hydroxyl radicals in an advanced oxidation process with salicylic acid trapping and liquid chromatography. *J Chromatogr A*, 796: 283–288.

Kilinc E, 2005. Determination of the hydroxyl radical by its adduct formation with phenol and liquid chromatography/electrochemical detection. *Talanta*, 65: 876–881.

Kramp F, Volz-Thomas A, 1997. On the budget of ·OH radicals and ozone in an urban plume from the decay of C5–C8 hydrocarbons and NO_x. *J Atmos Chem*, 28: 263–282.

Ma Z R, Zhao B L, Yuan Z B, 1999. Application of electrochemical and spin trapping techniques in the investigation of hydroxyl radicals. *Analytica Chimica Acta*, 389: 213–218.

McCabe T J, Maher J T, Actworth I N, 1997. Improved method for the estimation of hydroxyl free radical levels *in vivo* based on liquid chromatography with electrochemical detection. *J Chromatogr B*, 691: 23–32.

Ren X, Shao K, Miao G, 2001a. Determination of hydroxyl radical concentration in atmosphere. *China Environ Sci*, 21(2): 115–118.

Ren X, Shao K, Tang S, 2001b. Measurement of gas-phase ·OH using liquid phase scrubbing and high performance liquid chromatography. *Environmental Chemistry*, 20(1): 81–85.

Ren X, Wang H, Shao K, Miao G, Tang X, 2002. Determination and characteristics of ·OH radical in urban atmosphere in Beijing. *Environmental Science*, 23(4): 24–27.

Ren X, Brune W, Cantrell C, Edwards G, Shirley T, Metcalf A, Lesher R, 2005. Hydroxyl and peroxy radical chemistry in a rural area of Central Pennsylvania: Observations and model comparisons. *Journal of Atmospheric Chemistry*, 52(3): 231–257.

Salmon R A, Schiller C L, Harris G W, 2004. Evaluation of the salicylic acid-liquid phase scrubbing technique to monitor atmospheric hydroxyl radicals. *Journal of Atmospheric Chemistry*, 48: 81–104.

Thomas B, Muralikrishnan D, Mohanakumar K P, 2000. *In vivo* hydroxyl radical generation in the striatum following systemic administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine in mice. *Brain Res*, 852: 221–224.

Thompson A M, 1992. The oxidizing capacity of the earth's atmosphere: probable past and future changes. *Science*, 256: 1157–1165.

jesc.ac.cn