

Chemical Changes of Dissolved Sulfur Dioxide *In Vitro*

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*In Vitro*에서 SO₂ 수용액의 화학적 변화

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SUMMARY

Chemical changes of dissolved SO₂ were followed *in vitro* system. Varying concentrations of sulfite solution reached similar Eh values 20 hours after preparation. No further increase of Eh values occurred in acidic solutions. Eh values were more greatly increased in neutral solutions than in alkaline solutions, and slightly higher in light than in dark.

The decrease of measurable SO₂ content tended to be greater with increasing concentrations of sulfite, on exposure to light, and at higher pH values. A larger amount of hydrogen atoms was produced with higher concentrations of SO₂ and in neutral to alkaline solutions than in acidic solutions.

Introduction

Development of a reproducible model system simulating exposure of moist plant tissues to sulfur dioxide (SO₂) had been described in the previous paper⁽¹⁾. Lettuce leaf disks floated in aqueous solutions of SO₂ were shown to more severely discolored with higher concentrations of SO₂, at lower pH and on exposure to light. The bleaching action of dissolved SO₂ has been ascribed to the reduction of colors to colorless compounds by nascent hydrogen occurring in the course of its oxidation to sulfuric acid.^(1,2)

Phytotoxicity of SO₂ might be dependent upon the predominant species present in its aqueous solution.⁽³⁾ Based on the calculated oxidation-reduction potentials of SO₂, Puckett *et al.*⁽⁴⁾ stated

that all forms of dissolved SO₂ became increasingly better oxidizing agents as the pH of the medium was lowered. They suggested that the phytotoxic effect of dissolved SO₂ was associated with the destruction of chlorophyll by an irreversible oxidation process of this pollutant at lower pH levels.

Therefore it appears interesting to examine the primary chemical changes of dissolved SO₂ *in vitro*. The present investigation deals with the oxidation-reduction potential (Eh), measurable SO₂ content, hydrogen transfer, and pH change of aqueous SO₂ solution.

Materials and Methods

All experiments were conducted with unbuffered

red sodium sulfite solutions, one of testing media utilized for an investigation of the discoloration pattern of floated lettuce leaf disks, as described in the previous paper.⁽¹⁾

For SO_2 determination and Eh measurement, 50 ml of water or testing medium without leaf disks was placed in 9×1.5 cm petri dishes. A set of covered petri dishes were wrapped with aluminum foil to interrupt light, and another set of petri dishes were unwrapped. The former will be referred to as the dark condition and the latter as the light condition. These petri dishes were placed under 500 ft-c fluorescent light at $28-30^\circ\text{C}$, immediately after preparation.

Six stoichiometric concentrations of SO_2 —0, 200, 400, 600, 800, and 1,000 $\mu\text{g}/\text{ml}$ —were included as treatments. Initial pHs of the testing media were adjusted to 7 in all concentrations of SO_2 . As with other treatments, initial pHs of testing media—3, 5, 7, 9 and 11—were chosen with an SO_2 concentration of 600 $\mu\text{g}/\text{ml}$. The initial pH of the media was adjusted with sulfuric acid or sodium hydroxide for SO_2 determination and with hydrochloric acid or sodium hydroxide for Eh measurement.

Measurable SO_2 content was determined by the West and Gaeke's colorimetric method. Relative measurements of Eh values were performed with a Keithley Model 153 Microvolt-Ammeter. The reference electrode of a Model 12 Research pH meter (Corning Scientific Instruments) was connected to the cathode of a Microvolt-Ammeter and the platinum electrode to the anode. It usually took 1-10 minutes for the needle to be stabilized after dipping the electrodes in the sample solution.

Hydrogen transfer was tested with 2,3,5-triphenyl-tetrazolium chloride. Stoichiometric concentrations of SO_2 used in this experiment were 0, 2,000, 4,000, 6,000, 8,000, and 10,000 $\mu\text{g}/\text{ml}$. Each 2ml aliquot of SO_2 solution and equimolar tetrazolium chloride solution was placed in 18×150 mm culture tubes and well mixed. A set of capped tubes were wrapped with aluminum foil and another set of tubes were left unwrapped.

These tubes were placed under 500 ft-c fluorescent light at $15-20^\circ\text{C}$ for 20 hours. Precipitate was filtered under suction through pre-weighed No. 5C Toyo filter paper and dried to constant weight.

Results and Discussion

Oxidation-reduction potential is defined as a quantitative measure of free energy provided by oxidation or reduction in a reversible system and determined as the potential difference between that system and a standard electrode.⁽⁶⁾ Calibration of electrodes in absolute terms is very difficult, though relative measurements are quite simple. It is often hard to obtain reproducible results in Eh measurement⁽⁶⁾. Thus the data reported in the present investigation would be no more than comparative values.

As shown in Fig. 1, negative control and the lowest concentration of SO_2 had Eh values around +30 millivolts, while the remaining concentrations of SO_2 , similar Eh values around 0 millivolt immediately after preparation of media. However, Eh values increased up to around +70 millivolts in all SO_2 concentrations whether in

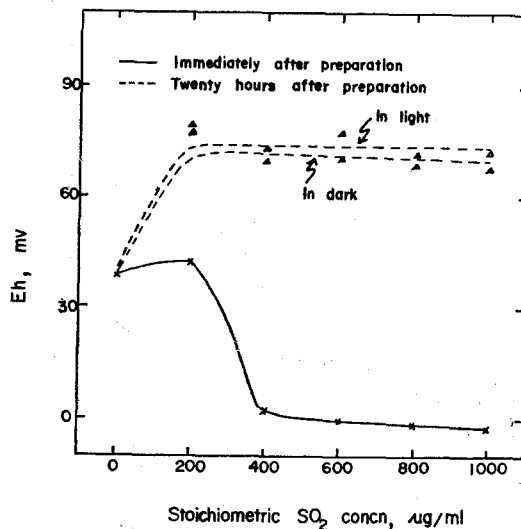


Fig. 1. Initial and final Eh values of various concentrations of SO_2 solution in light or dark condition. Initial pH was adjusted to 7 and final Eh was measured 20 hrs after preparation.

light or dark condition 20 hours after preparation of media.

The above results merely reveal that the ratio of oxidized forms of dissolved SO_2 to reduced ones increases in all SO_2 concentrations and in light or dark condition as time elapses.

When SO_2 concentrations were constant, Eh values were higher than 60 millivolts in initially acidic media, but were around 0 millivolt in initially neutral to alkaline media immediately after preparation. Twenty hours after preparation, no great differences of Eh values were found in acidic media; on the other hand, Eh values in neutral media were increased to the same extent as in acidic media. Eh values increased far more slowly in alkaline media. In neutral to alkaline media, Eh values were lower in dark than in light, the difference being greater as the pH became more alkaline (Fig. 2).

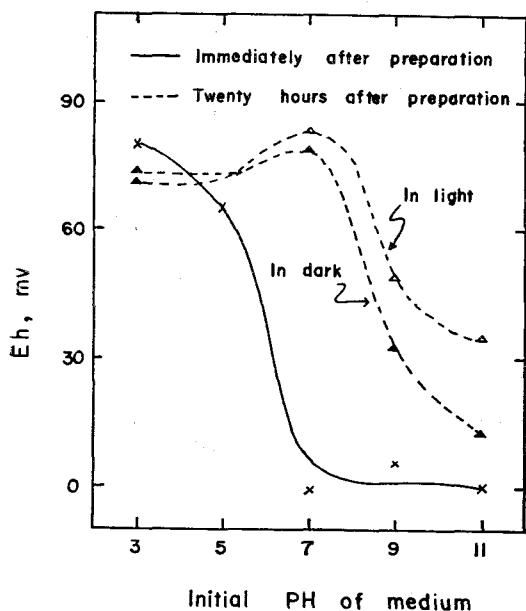


Fig. 2. Initial and final Eh values of various pHs of SO_2 solution in light or dark condition. Initial pH was adjusted to 3, 5, 7, 9 and 11 respectively, and stoichiometric SO_2 concentration was $600\mu\text{g/ml}$.

Under an assumption that dissolved SO_2 is oxidized to sulfate, the pattern of Eh changes appeared to be well coincided with that of pH changes, as described in the previous paper.⁽¹⁾

West and Gaeke's colorimetric estimation of SO_2 ⁽⁷⁾ is based on the fixation of this toxicant as disulfitomercurate (II) ion, $\text{Hg}-(\text{SO}_3)_2^{--}$. Accordingly, this method can be an indirect measure on the oxidation of dissolved SO_2 to sulfate.

Fig. 3 showed that the measurable SO_2 content of media was lower 20 hours after preparation than immediately after preparation. The decrease of measurable SO_2 content tended to be greater with increasing SO_2 concentration and on exposure to light. This result might be interpreted as an indirect evidence that higher concentrations of SO_2 or light made more rapid the progress of oxidation.

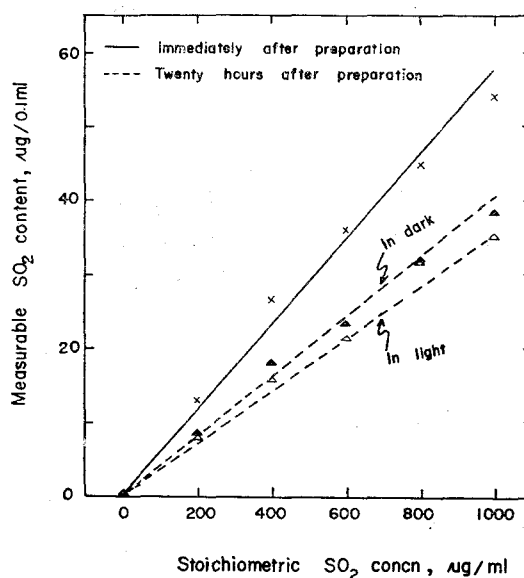


Fig. 3. Initial and final, measurable SO_2 content of various concentrations of sulfite solution in light or dark condition. Initial pH was adjusted to 7.

In light conditions, measurable SO_2 content was somewhat reduced in acidic media, whereas greatly reduced in alkaline media. Neutral media resulted in a moderate decrease of measurable SO_2 content. In dark conditions, reduction of measurable SO_2 content was slower, especially in alkaline media (Fig. 4). This result definitely implied that SO_2 species was converted to sulfate more rapidly in neutral to alkaline than in acidic ones.

Sulfurous acid captures oxygen from water

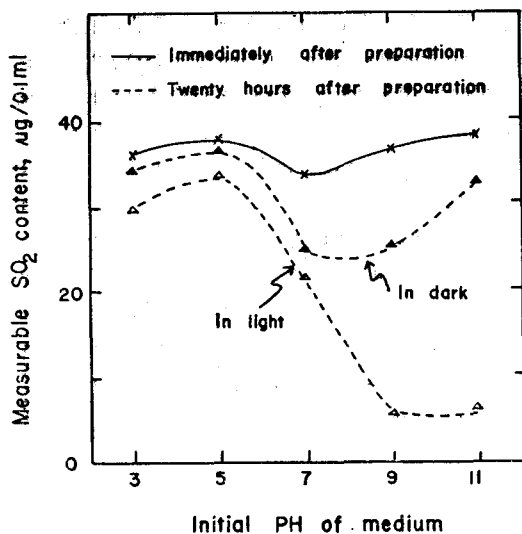


Fig. 4. Initial and final, measurable SO₂ content of various pHs of sulfite solution in light or dark condition. Stoichiometric SO₂ concentration was 600 µg/ml.

molecules and is oxidized to sulfuric acid, producing two hydrogen radicals.⁽⁸⁾ Discoloration of leaf disks floated in aqueous of SO₂ was attributed to hydrogen atoms being produced.⁽¹⁾ Accordingly, a means to detect the hydrogen transfer was needed.

Tetrazolium compounds have been widely used in biochemical reactions as hydrogen acceptors. It is one of the advantages of these compounds that they change color on reduction. As this chemical is itself colorless in aqueous solutions, but forms non-diffusible carmine red formazan upon reduction, triphenyl tetrazolium chloride was used for the purpose of confirming the hydrogen transfer.

As one proton and two electrons are transferred to each tetrazolium chloride molecule, it might be feasible to estimate the number of hydrogen atoms produced by weighing formazan precipitate. However, under this experimental condition, a sufficient amount of tetrazolium chloride solution to capture all hydrogen atoms produced was not added because the chemical we used had low solubility. Therefore, the amount of hydrogen atoms produced was not quantitated and only the

Table 1. Formazan precipitate produced from various concentration of SO₂ solution and equimolar tetrazolium chloride solution in light or dark condition. Initial pH of SO₂ solution was adjusted to 7. Precipitate was collected 20 hrs after preparation.

Stoichiometric SO ₂ concn (µg/ml)	Precipitate (mg)	
	In light	In dark
2,000	12.7	12.8
4,000	44.4	43.3
6,000	84.9	83.6
8,000	126.0	126.0
10,000	163.8	165.9

Table 2. Formazan precipitate produced from various pHs of SO₂ solution and equimolar tetrazolium chloride solution in light or dark condition. Stoichiometric concentration of SO₂ solution was 600 µg/ml. Precipitate was collected 20 hrs after preparation.

Initial pH of media	Precipitate (mg)	
	In light	In dark
3	6.7	5.5
5	15.9	13.9
7	86.8	84.2
9	88.5	83.2
11	74.8	71.3

precipitate was compared to approximate the tendency of hydrogen transfer among the treatments.

Table 1 indicated that precipitate was increasingly formed with higher concentrations of SO₂ and that light condition had no influence on the hydrogen transfer. At a constant concentration of SO₂, a greater amount of precipitate was formed in initially neutral to alkaline solutions than initially acidic solutions. A lesser amount of precipitate was formed at pH 3 than at pH 5 or at pH 11 than at pH 9. The amount of precipitate was slightly greater in light than in dark (Table 2).

The results of Fig. 4 and Table 2 indicated that discoloration of leaf disks might not be directly related to hydrogen transfer. Otherwise,

the formation of formazan precipitate might be dependent upon the pH of the solution regardless of the oxidizability of dissolved SO_2 . In addition, light seemed to have no great influence on the yield of hydrogen atoms. Thus more serious discoloration of leaf disks floated in acidic media remains questionable.

Gilbert⁽³⁾ suggested that a high pH value could, under certain conditions, allow SO_2 -sensitive species of bryophytes to survive by continually converting sulfur dioxide to a less toxic sulfate form. Since hydrogen atoms, in fact, were more abundantly produced at high pH compared with low pH (Table 2), there might be no direct correlation between leaf discoloration and hydrogen transfer against our previous assumption.⁽¹⁾

An alternative possibility on the bleaching action of SO_2 is the formation of colorless compound of SO_2 with the coloring matters.⁽⁸⁾ As mentioned previously, Puckett *et al.*⁽⁴⁾ related the increased toxicity of dissolved SO_2 at lower pH to the destruction of chlorophyll by an irreversible oxidation process. This might be another possibility of bleaching action.

In the previous report,⁽¹⁾ final pH of the medium served as an indirect evidence for the oxidation process of dissolved SO_2 . As discoloration of leaf disks appeared approximately 8-hours after floating in higher concentrations of SO_2 solution, the time course of the pH change of medium might well illustrate the progress of chemical changes of dissolved SO_2 .

When initial pH of medium was adjusted to 7 in all concentrations of SO_2 , control showed a very slight decrease of pH in 2 hours and no more change thereafter. The lowest concentration of SO_2 solution shows somewhat lower pH than higher concentrations 2 hours after preparation. However, increasing concentrations of SO_2 solution tended to exhibit decreasing pH values from 4 hours after start of the experiment. The pHs of media containing 200-1,000 $\mu\text{g}/\text{ml}$ of SO_2 represented almost identical patterns of change. All concentrations of SO_2 were consistently decreased and reached final pH during the 16-hour period. Apparently oxidation was progressed almost com-

pletely during an 8-hour period regardless of concentrations at pH 7. (Fig. 5).

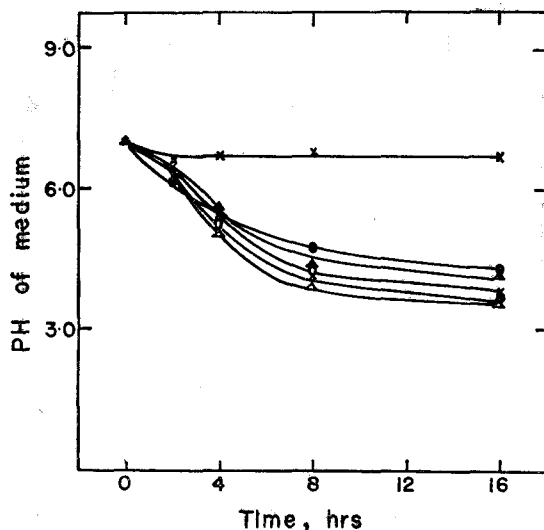


Fig. 5. pH change in various concentrations of SO_2 solution in light. Stoichiometric SO_2 concentrations were: 0(+—+), 200(·—·), 400(▲—▲), 600(×—×), 800(○—○), and 1,000(△—△) $\mu\text{g}/\text{ml}$. Initial pH was adjusted to 7.

Fig. 6 indicates that the pH change of media with a constant SO_2 concentration is entirely dependent upon initial pHs. Nearly no change was noticed at the lowest pH. Solution has reached final pH in 2 hours at pH 5 and in 8 hours at pH 7. Alkaline solutions also showed a gradual decrease of pH, even though their final pHs were still alkaline.

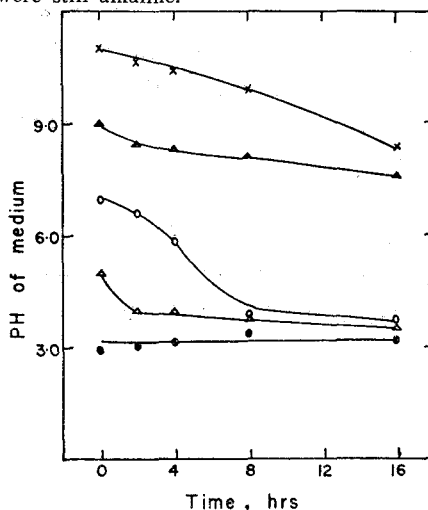


Fig. 6. pH change in various pHs of SO_2 solution in light. Initial pH was adjusted to 3, 5, 7, 9 and 11 respectively and stoichiometric SO_2 concentration was 600 $\mu\text{g}/\text{ml}$.

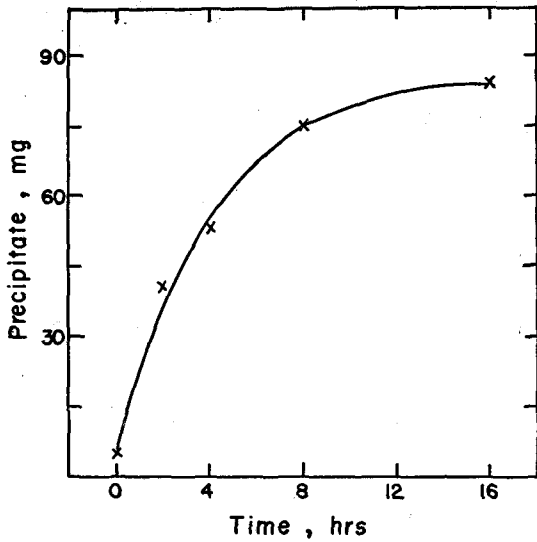


Fig. 7. Time course of formazan precipitate formation from 6,000 μ g/ml of SO₂ solution and equimolar tetrazolium chloride solution in light. Initial pH of SO₂ solution was adjusted to 7.

It seems that most hydrogen atoms are produced during an 8-hour period (Fig. 7). As discoloration of leaf disks was completed around 20 hours after floating in the previous experiment,⁽¹⁾ hydrogen atoms might not be directly contributed to the discoloration of leaf disks.

The present investigation was conducted to confirm our assumption derived from the study on discoloration pattern of leaf disks. However, the results are rather contradictory. Further studies would be needed to clarify the roles of hydrogen atoms and light for bleaching of plant leaf disks.

요 약

SO₂ 수용액의 화학적 변화를 *in vitro* system에서 조사하였다. 농도가 다른 수용액이 20시간 후

에 비슷한 Eh값에 도달하였으며 산성용액에서는 Eh값이 그 이상 증가하지 않았다. Eh값은 알카리성용액에서 보다는 중성용액에서 크게 증가하였고 암흑에서 보다는 광조건하에서 다소 높은 경향을 나타냈다.

유리상태의 SO₂함량은 아황산염의 농도가 증가하거나, 광에 노출한 경우, 또는 pH가 높은 때에 크게 감소하였다. 발생기수소는 고농도의 SO₂나 산성용액에서 보다는 중성 혹은 알카리성 용액에서 더욱 많이 생성되었다.



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