QUINOID GROUPS IN HUMIC ACIDS

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ABSTRACT


Quinoid groups of humic acids from various sources (soil, peat, brown and oxidized coals) can undergo reversible redox transformations, this being a fundamental property of this class of natural compounds.

Kinetic oxidation curves for reduced humic acids indicate the presence of various types of quinoid groups.

The specificity of a previously suggested technique for determining quinones in humic acids has been corroborated. Exhaustive carbonyl reduction by sodium borohydride combined with reductometric methods for determining quinones in alkaline media make possible a better estimation of the ketone content in humic acids.

When humic acids are reduced in an acid solution of tin chloride, rigorous conditions (4 h, 120°C, strong acid media) lead to an irreversible conversion of part of the quinone carbonyls and, as a result, the reaction proceeds nonstoichiometrically.

INTRODUCTION

The presence of quinone groups in the structure of natural humic acids and their quantitative determination have recently been receiving much attention in the literature*. To prove the presence of quinones, chemical data (Kukharenko and Yekaterinina, 1967; Yekaterinina and Kukharenko 1971; Mathur, 1972a, b; Rashid, 1972), IR-spectra (Mathur, 1972b, c) and experimental results of enzymatic degradation (Mathur, 1971) have been used, along with general considerations on the involvement of quinoid compounds in the formation processes of humic acids (Flaig, 1966).

Chemical evidence is chiefly based on reduction reactions, these being the most reliable and universal procedures for quinone determination. The elaboration of an acceptable quantitative method is, however, impeded by the insolubility of humic acids in water and most organic solvents. Quinone groups may

* When this manuscript was completed, we read a relevant paper by Schnitzer and Riffaldi (Soil Sci. Soc. Am. Proc., 36: 772, 1972). Agreeing in principle with these authors, we decided not to make any corrections herein.
be present in humic acid molecules as parts of various structural elements and may possess rather different redox properties. To eliminate the risk of incomplete determination of certain inert types of quinoid systems, one should use the most active reducing agents in the analytical procedure. Hence, the reduction reaction may also partly involve conjugated non-quinoid carbonyl groups which may also be present in humic acids.

Attempts to discover and quantitatively estimate quinone groups in humic acids with the aid of IR-spectra also encounter considerable difficulties. The spectrum region including quinone carbonyl absorption bands (1590–1680 cm⁻¹) encompasses numerous other chromophoric humic acid groups; as a result, reliable assignment of the bands found in this range becomes impossible.

Very significant in this respect is the failure of Mathur (1972b) to detect in the IR-spectra of humic substances discrete absorption bands caused by the expected presence of quinone carbonyls in their structure. Mathur's conclusions leave no hope for the direct use of IR-spectra to quantify amounts of quinones in humic acids.

At first sight, the possibility of mild enzymatic decomposition of humic acid macromolecules to fragments that can be identified and subsequent detection therein of native quinone groups from the initial acids appears to be rather challenging. As a matter of fact, such an attempt was recently made by Mathur (1971), who, by degradation of soil fulvic acids by enzymes of wood-destroying fungi, succeeded in isolating two compounds from a cultural medium, benzoquinone and 2-methyl-1, 4-naphthoquinone (15 and 10% of the starting material, respectively).

However, it is quite obvious that the said quinones could not have been parts of the fulvic acid molecules, their appearance in the enzymatic degradation products being due to the conversion and break-up of certain bonds that retain these cyclic systems within the initial molecules. Consequently, a part or, more probably, all of the quinone carbonyls of the isolated compounds were the result of enzyme effects or subsequent autooxidation of the primary decomposition products. Hence, these experimental results of enzymatic degradation of fulvic acids cannot be related to estimation of the quinone carbonyl content in the starting material. Previously, we had suggested a technique involving a reductometric semi-micro determination of quinone groups in humic substances (Glebko et al., 1970). The use of a rather active reducing agent, namely a solution of bivalent iron in alkaline triethanolamine (Fe-TEA method), allowed us to perform analyses within a short time period and under mild conditions. Numerous known quinones were used to show that the reaction with this reagent proceeds strictly stoichiometrically and is accompanied by the consumption of one reducing agent equivalent per quinone carbonyl, i.e., is completed at the stage of hydroquinone formation.

Similar results were obtained by us in using an alkaline solution of sodium stannite which, however, did not reduce anthraquinone and its hydroxy derivatives under the same analysis conditions (Vasilevskaya et al., 1971). For confident use of the method, it was necessary to prove that other groups, capable
of undergoing reduction with quinones, were absent in humic acids.

We tried to show this by taking advantage of the capability of quinones to regenerate readily from their reduced leucoforms when contacting atmospheric oxygen. For polycyclic quinones, similar processes are well known, being the basis of industrial methods for vat-dyeing and hydrogen peroxide production (Rodd, 1956; Donaldson, 1958; Cassidi and Kun, 1967). In the benzoquinone series, the oxidation of the corresponding hydroquinones is usually retarded; it accelerates only with increased pH values (James and Weisberger, 1938; Hathway and Seakins, 1955; Ettel and Pospisil, 1957).

No data were available showing that quinoid groups in humic substances could be arranged in a definite type of cyclic system. Hence, we realized that relevant theoretical discussion would be pointless and gave preference to experiment.

MATERIALS AND METHODS

The humic acid samples used in this study were characterized previously (Glebko et al., 1970). Known quinones were purified by vacuum sublimation or recrystallized till melting points were obtained corresponding to data in the literature.

Reduction by NaBH₄

Reduction of humic acids was carried out by a slightly modified Lindberg technique (Lindberg and Paju, 1953), which we had described previously (Glebko et al., 1972). An 0.1M solution of sodium borohydride in dimethylformamide (DMFA) was used, the reducer consumption for analytical determination being estimated gasometrically. Prior to the reduction procedure, the system and solutions were thoroughly flushed with pure argon. In preparative experiments, following complete reduction, the humic acids were precipitated by HCl. They were then washed free of mineral salts, freeze-dried and kept over P₂O₅. However, complete regeneration of quinoid groups took place only with oxidation by air at 40–50°C (see Table II).

Kinetic oxidation experiments

Portions of humic acids (400 mg) or of known quinones (1.0 mmole) and 10 ml of dry DMFA were placed in a vessel equipped with a gas inlet tube, a dropping funnel, and a side arm capped by a rubber septum. The vessel was flushed with pure argon, and an NaBH₄ solution in DMFA (2–3 ml) was added. The amount of NaBH₄ used to reduce all carbonyl groups contained in the humic acid samples was 200 % of the theoretical. Reduction continued for 15 h at room temperature with stirring by a magnetic mixer; during this time, the samples dissolved fully in the reagent. Ten ml of an acetic acid—DMFA mixture (1:1) was then dropped through a funnel; violent foaming occurred
indicating excess NaBH₄. Two portions of transparent solution (10 ml each) were sucked off with the aid of a long-needled hypodermic syringe and quickly transferred into special thermostatted oxidation cells (Fig. 1) filled with oxygen and connected with gas burettes. Oxidation continued for 6 h with vigorous agitation by a special magnetic mixer. For certain quinones and humic acids, this time period did not allow the reaction to be completed.

Fig. 1. Device for oxidizing reduced humic acids and quinones.

Regeneration of quinoid groups following reduction by acid tin chloride

Humic acids were reduced in sealed tubes in accordance with the procedure of Kukharenko and Yekaterinina (1967); the tin chloride content, however, was 5.5 and 30.0 mequiv./g of the acids. Following centrifugation of the cooled tubes, they were successively opened, and the aliquots (5.0 ml) of the supernatant liquid were titrated with an 0.1N iodine solution. The remaining portion in the tube was transferred to filters; the humic acid precipitates were thoroughly washed, first with 4N HCl, and then with water till complete removal of any traces of tin. Precipitates were then dried at 50°C and maintained in a dessicator over P₂O₅ for 7 days. The material obtained was used to determine the contents of regenerated quinone groups by the Fe-TEA method.

Oxidation of tin chloride solution in the presence of reduced humic acids

Preliminary reduction by tin chloride was run as follows: six equal weighed portions of humic acids (0.2 g) for each sample were reduced by 7 ml of an
acid SnCl₂ solution in sealed tubes according to the procedure of Kukharenko and Yekaterinina (1967). The first tube was centrifuged, and the remaining excess tin chloride in the aliquot (5.0 ml) of the supernatant liquid was determined by titration with an 0.1N iodine solution.

The rest of the tubes were then carefully opened, and air was blown through them at the rate of 20 ml/min. Control tests were run in similar tubes, each of these being filled with 7 ml of SnCl₂ solution; the solution concentration corresponded to the excess SnCl₂ remaining in the reduction experiments with the respective humic acids. After definite periods of time, the control test aliquots (5.0 ml) and centrifuged experimental solutions with the reduced humic acids were all titrated.

RESULTS AND DISCUSSION

Previously, we had performed repeated determinations of quinone groups in humic acids reduced by Fe-TEA and subsequently subjected to the action of air (Glebko et al., 1970).

The data obtained, however, could have involved errors caused by partial loss of semi-micro samples on their isolation from titrated solutions for a secondary determination.

In this work, we have subjected humic acids to preliminary treatment with excess sodium borohydride, which quantitatively reduced quinones to hydroquinones, and ketones to secondary alcohols (Gaylord, 1956; Hajos, 1971). The overall content of carbonyl groups in mequiv./g of humic acids was calculated with respect to the NaBH₄ consumed. Having been dried in air at 40–50°C, the reduced humic acids were analysed by the Fe-TEA method to determine the content of quinone groups. As is apparent from Table I, preliminary reduction of all carbonyl forms did not affect the content of the quinone groups recovered after air-oxidation.

Regeneration of the major portion of the quinone groups on contact of the humic acids with air proceeds rapidly (see Table II). Hence, it is possible to obtain preparations of humic acids with fully reduced quinoid carbonyls only under strictly anaerobic conditions. Consequently, all attempts to quantitatively elucidate the effect of reduction on the composition of the starting material, as, for example, with the aid of IR-spectra, are of doubtful validity.

To determine the relative rates of the above reaction, the reduced humic acids and known quinones were subjected to oxidation in a special device (Fig.1) with subsequent measurement of the volume of the oxygen consumed. In order that reduction and subsequent oxidation of humic acids should proceed in the solution, we used DMFA as the reaction medium, the decomposition of the excess NaBH₄ being achieved by adding an anhydrous acetic acid to give pH = 5.8–5.9. With such pH values, the starting unreduced humic acids and quinones absorbed virtually no oxygen, whereas at higher pH values they became markedly oxidized. The results from several such experiments are shown in Fig.2.
TABLE I

Contents of quinone and ketone groups in humic acids from various sources

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Source</th>
<th>Quinone groups (mequiv./g)</th>
<th>NaBH₄ used to reduce CO group (mequiv./g)</th>
<th>Quinone groups regenerated after reduction with NaBH₄* (mequiv./g)</th>
<th>Ketone groups** CO= (mequiv./g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Woodland soil</td>
<td>1.05</td>
<td>2.92</td>
<td>1.12</td>
<td>0.93</td>
</tr>
<tr>
<td>2</td>
<td>Peat</td>
<td>1.26</td>
<td>2.75</td>
<td>1.36</td>
<td>0.78</td>
</tr>
<tr>
<td>3</td>
<td>Brown coal</td>
<td>1.33</td>
<td>2.90</td>
<td>1.28</td>
<td>0.78</td>
</tr>
<tr>
<td>4</td>
<td>Brown coal</td>
<td>2.04</td>
<td>3.43</td>
<td>1.96</td>
<td>0.69</td>
</tr>
<tr>
<td>5</td>
<td>Weathered brown coal</td>
<td>2.51</td>
<td>3.95</td>
<td>--</td>
<td>0.72</td>
</tr>
<tr>
<td>6</td>
<td>Brown coal</td>
<td>1.91</td>
<td>3.72</td>
<td>1.80</td>
<td>0.91</td>
</tr>
<tr>
<td>7</td>
<td>Weathered brown coal</td>
<td>1.80</td>
<td>3.85</td>
<td>1.79</td>
<td>1.02</td>
</tr>
<tr>
<td>8</td>
<td>Weathered brown coal</td>
<td>2.98</td>
<td>4.06</td>
<td>2.86</td>
<td>0.53</td>
</tr>
<tr>
<td>9</td>
<td>Weathered brown coal</td>
<td>3.27</td>
<td>5.01</td>
<td>3.13</td>
<td>0.87</td>
</tr>
<tr>
<td>10</td>
<td>Weathered bituminous coal</td>
<td>3.37</td>
<td>5.18</td>
<td>3.35</td>
<td>0.90</td>
</tr>
<tr>
<td>11</td>
<td>Weathered hard coal</td>
<td>3.26</td>
<td>--</td>
<td>3.12</td>
<td>--</td>
</tr>
</tbody>
</table>

* Fe-TEA method.
** See text.
### TABLE II

Regeneration of quinone groups in humic acids reduced with NaBH₄

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Quinone group content (mequiv./g)</th>
<th>Initial acids</th>
<th>Preparations reduced with NaBH₄</th>
<th>Freezer-dried</th>
<th>24 h at 20°C in air</th>
<th>Successive heating in air at 50°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Freezer-dried</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>24 h at 20°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Successive heating in air at 50°C</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2.04</td>
<td>1.20</td>
<td>1.41</td>
<td>1.96</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>2.98</td>
<td>1.62</td>
<td>1.99</td>
<td>2.86</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>3.37</td>
<td>1.74</td>
<td>2.10</td>
<td>3.35</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. Oxidation rates of reduced humic acids and quinones. Curves: 1 = benzoquinone; 2 = duroquinone; 3 = 1,4-naphthoquinone; 4 = 9,10-anthraquinone; 5 = humic acids, sample 1; 6 = humic acids, sample 10; 7 = humic acids, sample 5; 8 = humic acids, sample 4.

The character of the curves for the oxygen absorbed by reduced humic acids indicates that these acids apparently contain oxidizable groups of various types. Thus, some react quite actively and disappear within a short time after absorption of 0.4–0.6 equiv. O₂/equiv. CO quinone, depending on the type of humic acid. The rest oxidize more slowly and at about the same rate, nearly equal to the hydroquinone oxidation rate for all the acid preparations tested.
The rate at which oxygen was absorbed by the reduced individual quinones varied widely and depended both on their structure and the nature of the substituents. Some quinones absorbed much more oxygen than the volume needed to transform hydroquinones into quinones. These deviations could be due to hydroxylation and dimerization of the quinones, as well as to the formation of hydrogen peroxide, which has been noted in several similar reactions. In the experiments with humic acids, however, the formation of hydrogen peroxide was not perceptible.

The above experiments were conducted with humic acid samples representing various natural sources. For all these diverse samples, as well as for the individual quinones of known structures, the analytical methods elaborated gave stable and reproducible results indicating the presence of a definite category of reducible carbonyls. The question as to whether all these carbonyls belong to typical quinones or whether other groups are involved in the reaction (e.g., quinone imines, quinone methides, etc.) may be solved only through further experimentation.

It has been reliably shown that functional groups, being chiefly and undoubtedly of quinoid origin and readily subject to reversible redox transformations, are contained in the molecules of natural humic acids. The number of such groups increases with the transition from soil humic acids to the humic acids of brown and weathered (oxidized) bituminous coals.

Previous elaboration of methods for determining acid functional groups has made it possible to estimate the cation-exchange properties of humic acids and the important role they play in geochemical processes and soil genesis. Presently, it has also become possible to make a simple assessment of the exchange redox capacity of these natural materials. In this connection, it appears important to elucidate the genuine role of quinone groups in humic acids and their complexes with transient-valency metals as regulators of the redox environment in soils and other natural formations. Here one should use the theoretical conceptions and abundant procedural experience accumulated in the study of synthetic electron-exchange polymers (Cassidi and Kun, 1967).

The methods elaborated can also help to make a new assessment of the content of ketone-carbonyl groups in humic acids. It is well known that the methods used for their determination, based on reactions leading to functional derivative ketones (oximes, hydrazones, etc.), are rather unreliable, because the participation of quinones in these reactions is not readily apparent.

When humic acids are reduced according to the method of Lindberg and Paju (1953), sodium borohydride is consumed in reactions with both quinones and ketone carbonyls. The ketone-carbonyl content may be found from the difference between the overall consumption of the reducing agent and the amount of quinones determined by reductometric titration according to the procedure suggested. In this case, one should only account for the different stoichiometry of the quinone and ketone reduction reactions (one and two equivalents of hydrogen per CO-group, respectively). If functional derivative ketones are obtained by the oximation technique of Fritz et al. (1959), for
instance, each carbonyl group corresponds to one titrant equivalent. Hence, the ketone-carbonyl content found (mequiv./g) will not correspond to the results obtained on reducing ketones with sodium borohydride. To standardize the data, the last figures should be divided by two.

The results of these calculations (see Table I) show that the ketone-carbonyl content in humic acids of different origins differ within a narrow range.

Recently, several papers have appeared (Kukharenko and Yekaterinina, 1967; Yekaterinina and Kukharenko, 1971; Mathur, 1972a, b; Rashid, 1972) in which the content of the quinone group in humic acids was estimated by reducing them with an acid solution of tin chloride (SnCl₂ method) according to the procedure of Kukharenko and Yekaterinina (1967). Although we have already had an opportunity to evaluate this method critically (Vasilevskaya et al., 1971), the appearance of these papers, involving a number of errors, obliged us to evaluate the method once again.

As is known, normal reduction of quinones takes place with the attachment of two electrons, formation of anion-radicals and transformation of the quinoid system to a benzenoid one (Fieser and Fieser, 1962). Thus, contrary to the above-cited authors, who all maintain that two equivalents of SnCl₂ are consumed by one of the quinone carbonyl groups, actually only one is involved.

With anthraquinone and its derivatives — and under extreme conditions with other quinones — tin chloride can cause further irreversible reduction of hydroquinones or their tautomers (Vorozhtsov, 1955; Rodd, 1956). This is due to the consumption of new portions of the reducing agent and the violation of the stoichiometry of the reaction. The possibility of such side reactions is not only considered possible but is postulated by the authors who use the SnCl₂-method. Thus, Yekaterinina and Kukharenko (1971, p.74) emphasized the complex multiform character of the action of tin chloride on humic acids, which is accompanied by transformations of quinoid carbonyls into phenolic, alcohol, and methylene groups. Mathur (1972b) attempted to find a corroboration for such a transformation, i.e., the appearance of alcohol and methylene groups in the IR-spectra of reduced humic acids.

If the action of the reagent during analysis really leads to such divergent results, i.e., to a different consumption of the reducing agent per one quinone carbonyl (two- or four-fold increase), such a reaction cannot serve as the basis for a quantitative analytical method.

We have attempted to clarify this issue and to establish to what extent the treatment with acid tin chloride affects the regenerability of quinone groups of reduced humic acids. The data in Table III show that, in this case, part of the quinone carbonyls is actually subject to irreversible transformations. The degree of these transformations depended on the excess of reducing agents, the consumption of tin chloride changing correspondingly. Thus, one may conclude that the reaction of SnCl₂ with humic acids is accompanied by the formation of various final products, i.e., it does not proceed stoichiometrically.

Apart from the factors causing increased consumption of the reagent, this method also involves some factors which have a directly opposite effect on
Regeneration of quinone groups after oxidation of humic acids reduced with SnCl₂ (effect of SnCl₂ quantities)

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>SnCl₂ (mequiv./g)</th>
<th>Quinone group contents* (mequiv./g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>taken for reduction</td>
<td>used on reduction</td>
</tr>
<tr>
<td>1</td>
<td>5.5</td>
<td>1.85</td>
</tr>
<tr>
<td></td>
<td>30.0</td>
<td>2.68</td>
</tr>
<tr>
<td>5</td>
<td>5.5</td>
<td>2.26</td>
</tr>
<tr>
<td></td>
<td>30.0</td>
<td>3.23</td>
</tr>
<tr>
<td>8</td>
<td>5.5</td>
<td>3.43</td>
</tr>
<tr>
<td></td>
<td>30.0</td>
<td>4.25</td>
</tr>
</tbody>
</table>

* All analytical operations were carried out in argon.
** Fe-TEA method.

The action of an acid solution of tin chloride on humic acids takes place under heterogeneous conditions and in the absence of agitation; it is consequently difficult to ensure complete and uniform interaction of the reagent with the material being analysed. Hence, it is no wonder that in the experiments of Mathur (1972a, b) known quinones were reduced by only 46–66% (the sole exception being vitamin K-S-II), whereas 1,2-dihydroxyanthraquinone and 2-carboxyanthraquinone were not reduced even when subjected to repeated treatments.

Complete and strictly stoichiometric reduction of known quinones of different structures using analytical procedures still remains the only reliable criterion for a method of determining quinone groups in humic acids. Consequently, Mathur's assertions about the effectiveness of the SnCl₂ method are perplexing.

An unacceptable procedure in the SnCl₂ method is the filtration in air of the tube contents following reduction. SnCl₂ solutions are rapidly oxidized by air, especially in the presence of large amounts of HCl (Mazeyewski and Kutner, 1966). The presence of a precipitate of reduced humic acids considerably retards the process, this ostensibly being due to the known antioxidizing influence of phenolic compounds. Table IV shows the oxidation rates of tin chloride in acid solutions in the presence and absence of preliminarily reduced humic acids. The reduction of certain humic acid samples by tin chloride is accompanied by the formation of coloured degradation products soluble in aqueous acids. The inhibiting effect of these preparations was especially pronounced.
TABLE IV

Effect of reduced humic acids on air-oxidation rate of SnCl₂ solutions

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Concentration of SnCl₂ (mequiv./g)</th>
<th>Sample 1</th>
<th>Sample 5</th>
<th>Sample 8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A*</td>
<td>B**</td>
<td>A*</td>
</tr>
<tr>
<td>0.0</td>
<td></td>
<td>0.145</td>
<td>0.143</td>
<td>0.131</td>
</tr>
<tr>
<td>0.5</td>
<td></td>
<td>0.116</td>
<td>0.131</td>
<td>0.103</td>
</tr>
<tr>
<td>1.0</td>
<td></td>
<td>0.085</td>
<td>0.112</td>
<td>0.075</td>
</tr>
<tr>
<td>2.0</td>
<td></td>
<td>0.022</td>
<td>0.075</td>
<td>0.019</td>
</tr>
<tr>
<td>2.5</td>
<td></td>
<td>0.007</td>
<td>0.058</td>
<td>0.006</td>
</tr>
<tr>
<td>3.0</td>
<td></td>
<td>0.000</td>
<td>0.042</td>
<td>0.000</td>
</tr>
</tbody>
</table>

* A: pure SnCl₂ solution (control).
** B: SnCl₂ solution with reduced humic acids.

These results show that the use of blank test data not only does not eliminate the oxidation effect of SnCl₂ solutions at filtration, but, on the contrary, leads to badly distorted results for the contents of quinone groups in humic acids.

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REFERENCES


