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Short communication

Determination of OH groups in humic acids using methylation with dimethylsulfate

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ABSTRACT

The methylation of humic acids (HA) with dimethylsulfate in acetone and methanol followed by the iodometric determination of the methoxy groups (Zeisel reaction) were applied to determine the contents of –OH groups in solid samples of HA of different origins. For the coal- and peat-derived HA samples, the contents of –OH groups determined after methylation in acetone ranged from 6.6 to 8.7 mmol/g, whereas the contents of –OH groups determined after methylation in methanol ranged from 4.0 to 5.0 mmol/g. These differences may be related to the content of carboxylic groups in the HA molecule that were not methylated in methanol, as confirmed by a comparison with results of conventional titrimetric determinations. Observed differences were interpreted as results of different polarity of both solvents and alkalinity of the reaction mixture during the methylation. The contents of alcoholic groups as well as some other minor –OH groups can be estimated using the –OH group contents obtained after methylation in both solvents together with the results of the conventional determinations of acidic functional groups. A repeatability of the –OH groups determination as estimated from a series of triplicate analyses of different HA samples ($n = 7$) was in range of 0.15–0.73 mmol/g and 0.08–1.06 mmol/g (standard deviations) for methylation in acetone and methanol, respectively. Thus, the average repeatability of the –OH groups determination was estimated to be 0.38 and 0.50 mmol/g for methylation in acetone and methanol, respectively.

1. Introduction

The diversity of functional groups and a complex structural skeleton of humic substances (HS) make their fractionation and structural characterization by common approaches rather difficult. Therefore, the development of new analytical approaches or combination of different known techniques is still desirable. One of important tools in the characterization of HS represents derivatization. The aim of derivatization is a modification of certain properties of HS in a desired way, e.g. for easier detection, elimination of hydrogen bonds or reduction of HS polarity [1]. The most frequent functional groups occurring in the structure of HS are –OH groups either alone standing (phenolic, alcoholic, enolic –OH) or as a part of other functional groups (carboxylic –COOH, hydroperoxidic –O–OH). The derivatization of –OH groups in HS is mainly done using methylation as a relatively common way of acidic hydrogen substitution in –OH groups [2,3]. Ricca et al. [4] reported on the methylation of –OH groups in humic acids (HA) obtained from Leonardite using the mixture CH_3I/Ag_2O in methanol and dimethylformamide. The main goal of methylation was the removal of strong hydrogen bonds in HA leading to the formation of intra- and intermolecular aggregates disabling their dissolution in organic solvents and thereby the determination of their molecular mass. The methylated products were well soluble in organic solvents, which enabled their analysis by spectral techniques (IR, ^1H and ^{13}C NMR). The IR spectra of the methanolic product showed that all –COOH groups were derivatized to methyl esters (absorption band at 1730 cm^{-1}). Other –OH groups were methylated only partly (absorption at 3450 cm^{-1}). When dimethylformamide was used as a solvent both carboxylic –OH as well as other –OH groups were methylated (no absorption at 3450 cm^{-1}, strong absorption at 1730 cm^{-1}). The analysis of HA by ^{13}C NMR after methylation by diazomethane and alkali hydrolysis of methylated products was the subject of the paper published by Sachs et al. [5]. In the first step, the –COOH groups were blocked by methylation in the form of methylesters, whereas the phenolic –OH groups were blocked as methylethers, as confirmed by NMR. In the second step, the selective hydrolysis of methylesters in alkali solution was carried out. Thereby the –COOH

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groups were again unblocked. The methylethers did not hydrolyzed under given conditions. This procedure enabled to study the influence of phenolic OH groups on the complexation behaviour of HA. The methylation of HA in situ with tetramethylammonium hydroxide (TMAH) was applied to study the HA structure by conventional flash pyrolysis [6]. It was stated that the methylation protected –COOH groups and aliphatic chains during the pyrolysis against the cleavage and chemical reactions even with an excess of sulphur. The similar technique was used for the study of distribution of HS and lipids in two different sewage sludges [7]. HA were investigated by elemental analysis, IR and thermally assisted hydrolysis and methylation coupled to GC–MS (THM–GC–MS). The influence of methylation with diazomethane on molecular mass distribution in aqueous fulvic acids was investigated by electrospray mass spectrometry [8]. The aim of methylation was to suppress the influence of acidic hydrogens in the formation of aggregates of fulvic acids and to eliminate the formation of higher charged ions during the MS analysis. The successfulness of methylation was monitored by IR.

The methylation with dimethylsulfate [1–3] or acetylation with acetic anhydride [1,3] have been applied often for the determination of –OH groups (or "total –OH") contents in HA. When dimethylsulfate is used as methylating agent the sample is repeatedly let to react with it in alkali solution. The formed precipitate is analyzed after acidification on the presence of methoxy groups by Zeisel reaction. It is supposed usually that only phenolic and enolic –OH groups react with dimethylsulfate. The possible problems with side reactions of formed sulfate in aqueous alkali solution can be eliminated by using acetone [2] or methanol [1] as solvent instead of water.

In this work, the methylation with dimethylsulfate was employed for the determination of the –OH groups contents in HA. Significant differences were observed when acetone and methanol were used as solvents during the methylation—a possible explanation of this phenomenon is given in the paper. It was also shown that some useful additional parameters, such as amounts of alcoholic or some minor –OH groups, can be estimated from the –OH groups content determined by the proposed method and other parameters determined by conventional titrimetric procedures.

2. General principle of the determination

The procedure for the determination of –OH groups consists of two steps:

1. The substitution of hydrogen in –OH groups by methyl groups ($-CH_3$).
2. The cleavage of the methoxy groups ($-OCH_3$) by hydroiodic acid under formation of gaseous methyl iodide, which is trapped in bromine water and determined by iodometric titration as released iodine (Zeisel reaction).

The substitution of hydrogen in –OH group is realized by reaction between HA and dimethylsulfate $(CH_3)_2SO_4$, which can be described by the following equations [2]:

$$ROH + NaOH \leftrightarrow RO^- + Na^+ + H_2O$$

$$RO^- + CH_3{-}O{-}SO_2{-}O{-}CH_3 \rightarrow R{-}OCH_3 + CH_3{-}O{-}SO_2{-}O^-$$

The methylation reaction can be carried out in the system of acetone with K_2CO_3 or methanol with K_2CO_3. Because none of these solvents is preferred in literature, the methylation was done in both solvents in this work in order to investigate their influence on the determination of the –OH groups in HA.

Table 1
Solid HA used in this work

Sample	Origin and basic characteristics
HA1	HA from oxihumolite[a] from North-Bohemian coal basin, mining site Václav (1), content of H_2O 7.1%, ash content 2.8%
HA2	Refined HA from HA1, content of H_2O 7.5%, ash content 1.8%
HA3	HA from oxihumolite[a] from North-Bohemian coal basin, mining site Václav (2), content of H_2O 5.7%, ash content 7.7%
HA4	HA from oxihumolite[a] from North-Bohemian coal basin, mining site Vršany, content of H_2O 7.3%, ash content 11.2%
HA5	HA from peat from South-Bohemian basin Třeboň, locality Braná, content of H_2O 7.0%, ash content 7.1%
HA6	HA from peat from Bohemian Forest, locality Světlík, content of H_2O 6.6%, ash content 5.1%
HA7	HA from young brown coal oxidized by HNO_3, mining site Družba, Sokolov Basin, content of H_2O 6.2%, ash content 2.0%

[a] Weathered, naturally oxidized young brown coal.

The determination of $-OCH_3$ groups by the Zeisel reaction can be described by the following equations [3]:

$$R{-}OCH_3 + HI \rightarrow ROH + CH_3I$$

$$CH_3I + 6Br_2 + 5H_2O \rightarrow HIO_3 + 12HBr + CO_2$$

$$2HIO_3 + 10KI + 5H_2SO_4 \rightarrow 6I_2 + 6H_2O + 5K_2SO_4$$

$$I_2 + 2Na_2S_2O_3 \rightarrow 2NaI + Na_2S_4O_6$$

The content of metoxy groups in HA samples found by the Zeisel reaction should be recalculated on the –OH content (in mmol/g of the dried- and ash-free sample).

3. Experimental

3.1. Materials and reagents

Various kinds of solid HA were prepared from young brown coals and other materials (peats) by alkaline extraction and acid precipitation, as described in details elsewhere [9]. The solid samples were used without an additional pre-treatment except of grinding. A list of the HA samples used in this work is given in Table 1 together with their basic characterization. Stock solution of $Na_2S_2O_3$ (0.1 mol/l) was prepared from reagent-grade product (Lach-Ner, Neratovice, Czech Republic) and standardised by a conventional titrimetric procedure. The solutions were prepared in deionised water from the system Demi Ultra 20 (Goro, Prague, Czech Republic) utilising reverse osmosis and mixed-bed ion-exchange for the water purification. Bromine water for the Zeisel reaction was prepared as follows: Dissolve 100 g of potassium acetate (Lach-Ner) in 1000 ml of solution containing 900 ml of glacial acetic acid (Penta, Chrudim, Czech Republic) and 100 ml of acetic acid anhydride (Aldrich, Steinheim, Germany). Then dissolve 5 ml of bromine (Aldrich) in 145 ml of the potassium acetate solution. This solution must be prepared daily fresh. Water-free K_2CO_3 was prepared by ignition of the reagent-grade product (Lach-Ner) over the Bunsen burner in a Pt vessel for 15 min and stored in a desiccator over silica gel. Sodium acetate buffer was prepared by dissolving 220 g sodium acetate (Lach-Ner) in 1 l of deionized water. Organic solvents (acetone, methanol) were of reagent-grade quality, obtained from Penta, Chrudim, Czech Republic, dimethylsulfate (99%) was obtained from Aldrich.

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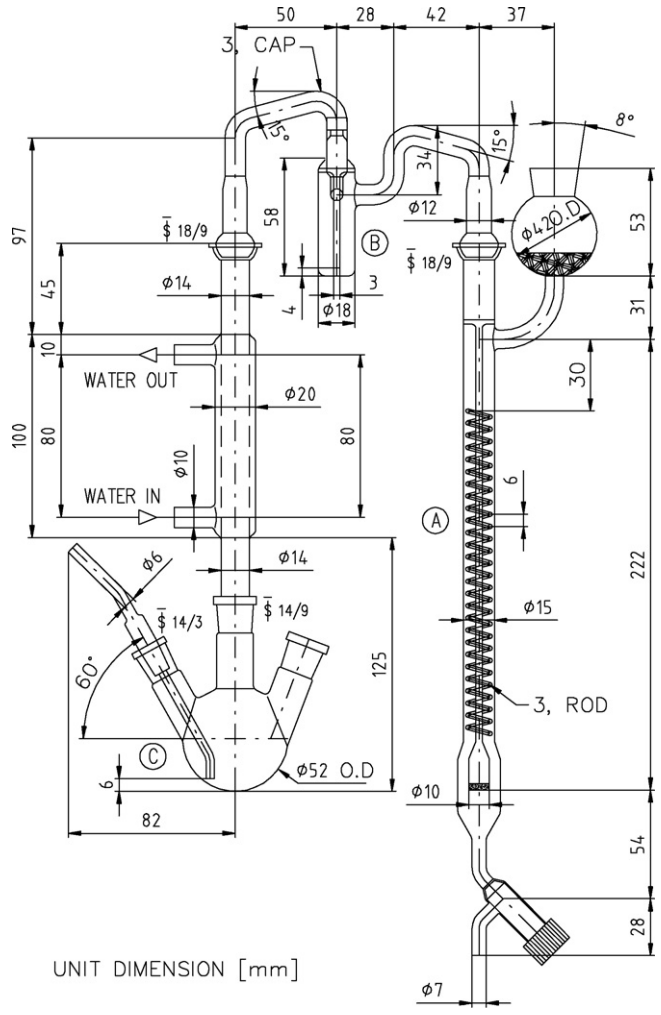

Fig. 1. Apparatus for the Zeisel reaction. (A) Absorber filled with bromine water; (B) iodine vapour trap; (C) reaction flask with water cooler.

3.2. Apparatus and procedures

Procedure for methylation with dimethylsulfate: 1 g of HA was refluxed for 24 h with 14 ml of dimethylsulfate and 24 g of water-free K_2CO_3 in 40 ml acetone or methanol in 100 ml rounded flask under water cooler. The reaction mixture was continuously stirred by magnetic stirrer to avoid a secret boiling. Then the organic solvent was removed from the reaction mixture by distillation under normal pressure using a water bath. The reaction mixture was acidified to pH 2 with HCl (ca. 2 mol/l) to precipitate solid HA. The precipitated HA was dried overnight at 40 °C in an oven. Dry HA was re-methylated three times with 8 ml of dimethylsulfate and 16 g of water-free K_2CO_3 in 40 ml acetone or methanol.

Procedure for the Zeisel reaction: the apparatus for the Zeisel reaction was constructed as described in the literature [10,11] with slight modifications only (Fig. 1). In principle, the apparatus consits of an absorber (A) filled with bromine water and of a distillation part containing an iodine vapour trap (B), water cooler and a reaction flask (C) with the arm for nitrogen supply. The Zeisel reaction was carried out as follows: add 3 ml of deionized water to the iodine vapour trap and 10 ml of bromine water to the absorber. Attach the absorber to distillation part. Weigh 50 mg of HA sample into the reaction flask, add 6 ml of hydroiodic acid (57%, Aldrich) and attach the reaction flask to the apparatus. Connect the side arm of the reaction flask to nitrogen source and adjust the flow rate of nitrogen through iodine vapour trap to 2 bubbles/s. Heat the reaction flask for 3 h at 150 °C. Then transfer the content of the absorber to the vial containing 15 ml of sodium acetate buffer. Eliminate the excess of bromine by adding a few drops of formic acid (check with the methyl red indicator [12]). When the solution is free of bromine add 5 ml of sulphuric acid (ca. 2 mol/l) and titrate the released iodine with 0.1 mol/l sodium thiosulfate. Simultaneously, the blank experiments with all reagents were carried out.

It should be taken into account that the methylation reaction increases the weight of the sample. Therefore, the conversion of percentage of methoxy groups in methylated product to percentage of –OH groups in the original sample must be done as follows:

$$\text{percentage of } -\text{OH in original sample} = \frac{1700(Y_2 - Y_1)}{3100 - 14Y_2}$$

where Y_1 is the percentage of $-OCH_3$ in the original sample and Y_2 is the percentage of $-OCH_3$ in the methylated product.

Conventional titrimetric procedures were used for the determination of acidic functional groups in HA, namely the barium hydroxide method for the determination of total acidity and the calcium acetate exchange method for the determination of carboxylic groups, respectively [13]. For comparison, the content of carboxylic groups was determined also from the acid–base titration curves [14] using the method of Ritchie and Perdue [15].

Table 2
Determination of the OH groups content using methylation in acetone $(OH)_{ac}$ and in methanol $(OH)_{meth}$ with corresponding standard deviations (S.D.) calculated from triplicate analyses

Sample	$(OH)_{ac}$ (mmol/g)	S.D. (mmol/g)	$(OH)_{meth}$ (mmol/g)	S.D. (mmol/g)
HA1	8.4	0.26	4.9	0.20
HA2	8.4	0.15	4.0	1.06
HA3	8.7	0.64	5.0	0.35
HA4	8.7	0.73	4.4	0.65
HA5	6.9	0.25	4.1	0.50
HA6	6.6	0.37	4.7	0.68
HA7	7.1	0.29	4.6	0.08

Values were re-calculated on the water- and ash-free samples.

4. Results and discussion

The –OH groups contents and corresponding standard deviations from triplicate analyses obtained by methylation with dimethylsulfate in acetone $(OH)_{ac}$ and in methanol $(OH)_{meth}$ followed by the Zeisel reaction are given in Table 2. It is distinct that the values of $(OH)_{ac}$ are almost two times higher in comparison with $(OH)_{meth}$. As already mentioned, the difference in using acetone or methanol for determination of the –OH groups is not emphasized in literature [2,3]. The possible explanation of this difference consists in a different reaction environment, especially regarding the alkalinity of the reaction mixture and polarity of the used solvents.

Table 3
Determination of carboxylic functional groups—a comparison of various methods

Sample	Ca acetate method (mmol/g)	From titration curve[a] (mmol/g)	From the difference between $(OH)_{ac}$ and $(OH)_{meth}$ (mmol/g)
HA1	4.9	4.5	3.6
HA2	4.1	5.0	4.4
HA3	4.0	4.1	3.7
HA4	3.7	4.4	4.3
HA5	2.7	3.4	2.8
HA6	2.5	3.2	1.9
HA7	3.0	3.3	2.5

[a] Calculated according to the procedure of Ritchie and Perdue [15] from the base consumption up to pH 8.

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Table 4
Contents of various functional groups in HA

Sample	Total acidity[a] (mmol/g)	Phenolic groups[b] (mmol/g)	TAB groups[c] (mmol/g)	Alcoholic groups[d] (mmol/g)
HA1	9.4	4.5	1.0	0.3
HA2	9.3	5.2	1.0	<0.1
HA3	9.6	5.6	1.0	<0.1
HA4	10.0	6.3	1.3	<0.1
HA5	8.1	5.4	1.2	<0.1
HA6	7.4	5.0	0.9	<0.1
HA7	7.6	4.6	0.5	<0.1

[a] Barium hydroxide method.
[b] Determined by the conventional procedures as a difference between the total acidity and the content of carboxylic groups.
[c] Sum of tertiary allyl and benzyl OH groups determined as a difference between the total acidity and $(OH)_{ac}$.
[d] Calculated from the difference between $(OH)_{meth}$ and the phenolic group content.

Due to higher polarity of methanol in comparison to acetone the solubility of K_2CO_3 in the reaction mixture was higher, and consequently the alkalinity of the reaction mixture was higher, too. Increased alkalinity of the reaction mixture led to the methylation of phenolic, alcoholic and hydroperoxidic –OH groups (–O–OH) only in methanol. When acetone was used as solvent, on the other hand, the methylation of carboxylic groups (–COOH) together with the previously mentioned –OH groups took place. It was found in early studies on the methylation of HA with dimethylsulfate that the carboxylic groups are not methylated in strongly akaline solutions [16,17], but they are methylated under mildly alkaline conditions [18], which supports the above explanation. Thus, the difference between $(OH)_{ac}$ and $(OH)_{met}$ could give the content of –COOH groups in the HA molecule. The comparison of the contents of carboxylic groups as determined by three different procedures is given in Table 3. As can be seen, the obtained results for the three different procedures are in a reasonable agreement, taking into account quite different principles of the determinations. The values of –COOH content calculated as the difference between $(OH)_{ac}$ and $(OH)_{met}$ are mostly in the interval allocated by two remaining techniques.

As can be deduced from literature [1], some minor –OH groups (tertiary, allyl and benzyl) are not methylated with dimethylsulfate. Hence, the content of these groups can be estimated from the difference between the total content of –OH groups (total acidity) and the $(OH)_{ac}$ value—see Table 4. Moreover, the content of alcoholic groups can be calculated as a difference between $(OH)_{meth}$ and the content of phenolic groups, as determined by the titrimetric procedure (Table 4).

5. Conclusions

The methylation of HA with dimethylsulfate in acetone and methanol was applied to various solid samples of HA of different origins. It was found that the content of –OH groups determined after methylation in acetone is higher then the content of –OH groups determined after methylation in methanol. This difference may be related to the content of carboxylic groups in the HA molecule, as confirmed by a comparison with results of conventional titrimetric determinations. Observed differences were interpreted as results of different polarity of both solvents and alkalinity of the reaction mixture. The contents of alcoholic groups as well as some other minor –OH groups can be estimated using the –OH group contents obtained after methylation in both solvents together with the results of the conventional determinations of acidic functional groups. A repeatability of the –OH groups determination as estimated from a series of triplicate analyses of different HA samples ($n = 7$) was in range of 0.15–0.73 mmol/g and 0.08–1.06 mmol/g (standard deviations) for methylation in acetone and methanol, respectively. Thus, the average repeatability of the –OH groups determination was estimated to be 0.38 and 0.50 mmol/g for methylation in acetone and methanol, respectively.

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