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# Evaporative Light Scattering: A Novel Detection Method for the Quantitative Analysis of Humic-like Substances in Aerosols

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The chemical composition of organic atmospheric aerosols is only poorly understood. Although a significant fraction of organic aerosols consists of humic-like substances (HULIS), only little is known about this class of compound, and accurate quantification remains difficult, partly due to the lack of appropriate standards. Here, evaporative light-scattering detection (ELSD) was applied for the first time to quantify water-soluble HULIS in aerosol particles smaller than 1  $\mu\text{m}$ . This detection method was shown to be suitable for the quantification of compounds with unknown structures and lacking appropriate quantification standards. As compared to organic carbon determination of isolated HULIS, no organic carbon/organic mass (OC/OM) conversion factor needs to be applied with ELSD and therefore eliminates this significant uncertainty factor of the OC/OM method, which is frequently used to quantify HULIS. Solid-phase extraction and size-exclusion chromatography were applied to separate inorganic ions and low molecular weight compounds from HULIS before ELSD quantification. The ELSD itself provides an additional separation step where low volatility HULIS are separated from high volatility, small compounds. Electrospray ionization mass spectrometry was used to identify the molecular weight range of the compounds quantified with ELSD. The most intensive peaks were in the range of  $m/z$  200–500, with some masses up to  $m/z$  800. We showed that UV detection using fulvic acid as surrogate quantification standard underestimates the HULIS concentration by a factor of 1.1 to 2.5, which is in agreement with earlier studies. During a 6 week winter 2005–2006 campaign at a suburban site near Zurich, Switzerland, an average of 1.1  $\mu\text{g}/\text{m}^3$  HULIS was found, which is about 4–6% of the total particle mass smaller than 1  $\mu\text{m}$  (PM<sub>1</sub>) and 10–35% of the organic matter in PM<sub>1</sub>.

## Introduction

Hundreds of organic compounds have been identified in organic atmospheric aerosols so far (1); however, together

they constitute less than 10–20% of the organic carbon (OC) of urban and rural aerosol (2, 3). The unresolved mass can be partially attributed to bacteria (4), fungal spores (4), proteins (5), cellulose (6), and humic-like substances (HULIS) (7). HULIS are a class of compounds with properties (acidity, UV–vis absorbance, fluorescence, molecular size range) similar to humic substances (8), which are found in water, sediments, and soil. The term HULIS describes a large number of polydisperse, heterogeneous, and relatively polar organic compounds that are water soluble. They have many functional groups such as acids, alcohols, ketones, organosulfates, nitrogen-containing functional groups, as well as aromatic and aliphatic moieties. The molecular weight distribution of HULIS goes up to 500–700 Da as determined by mass spectrometry, with average masses in the range of roughly 200–300 Da (9–12). A comprehensive recent review on HULIS can be found in Graber and Rudich (13).

For separation of HULIS from other organic aerosol components, ultrafiltration (8), capillary electrophoresis (14, 15), solid-phase extraction (SPE) (7, 16, 17), ion exchange chromatography (18), reversed-phase chromatography (11, 19), and size-exclusion chromatography (SEC) (10, 12, 15) have been used. Because of the heterogeneous chemical properties of HULIS, the characterization of the HULIS fraction is often based on separation and/or detection methods rather than on unambiguous (chemical) criteria.

The quantification of the water-soluble HULIS has been attempted with several techniques. These analyses have often been adapted from experiences made in humic substance research (20, 21). Studies investigating the quantity of HULIS in atmospheric aerosols can be found in refs 7, 8, 18, 22, 23, and 24. An extensive overview about HULIS in general and quantification methods is given in a recent review by Graber and Rudich (13). The fraction of HULIS in water-soluble organic carbon (WSOC) is in the range of 15–60%.

In combination with the previously mentioned separation techniques, optical detection methods such as UV (8, 10, 22) or fluorescence (19) are often used for quantification. These optical methods require the use of appropriate quantification standards with identical optical properties. Because such standards are not available for HULIS, compounds with similar absorption properties such as humic or fulvic acids are used, resulting in large uncertainty. An alternative approach to determine the concentration of HULIS is the determination of organic carbon content in HULIS (e.g., refs 8, 18, 23, and 24). Limbeck et al. recently presented a semi-online method for HULIS separation and OC quantification (7). However, to determine the total organic mass of HULIS from its organic carbon content, a conversion factor is required that has to be estimated (1.4–2.1 depending on location and season (25)), which introduces a large uncertainty. In addition, there is considerable disagreement between different thermal and optical OC determination methods, which results in an additional uncertainty factor of about 2–3 for the determination of the OC concentration (26). However, thermal and optical OC methods have the advantage that they allow for a determination of a carbon mass balance and that most of these methods are very sensitive.

In this study, we used a combination of SPE and SEC coupled with ELSD and ESI-MS for the separation and quantitative determination of the water-soluble HULIS fraction of aerosols collected at a suburban site in Switzerland. This paper introduces a detection method that is well-known in polymer analyses but is new to atmospheric science: evaporative light-scattering detection (ELSD) (27–29). Light-

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scattering detection has the beneficial attribute of being dependent only on the analyte concentration. Quantification of organic compounds with this universal detection method is independent of the limitations of spectroscopic methods because it is independent of optical properties such as number and type of chromophores of the analytes. In contrast to organic carbon analysis (7, 8, 18, 23, 24), the ELSD method does not need to assume the difficult-to-estimate organic carbon/organic mass conversion factor. Furthermore, ELSD quantification is not disturbed by mobile-phase changes in separation methods, which often cause a baseline shift for many detection methods (e.g., in gradient elution). The ELSD consists of three steps: (i) nebulization of the column effluent with the assistance of a nebulizing nitrogen gas flow. (ii) Evaporation of solvent and modifiers as well as volatile analyte compounds in a heated evaporation tube leaving the lower volatility compounds as aerosols. The evaporation tube acts like a thermodenuder frequently used in atmospheric sciences. (iii) Light scattered by the aerosol particles at the end of the thermodenuder (evaporation tube) is detected by a photomultiplier. It has been shown in the literature that a universal calibration is valid for a wide range of analytes, being largely independent of chemical properties of the analytes (30, 31).

## Experimental Procedures

**Aerosol Sampling.** Aerosol particles were sampled in Dübendorf, Switzerland (430 m asl) between December 15, 2005 and January 26, 2006. The location was directly next to a site of the Swiss National Air Pollution Monitoring Network (NABEL). The surrounding area of Dübendorf is densely populated and includes industries and heavy traffic roads within 150 m and highways within 750 m. This location is considered a suburban site. Weekly samples of particles smaller than 1  $\mu\text{m}$  in diameter (PM<sub>1</sub>) were collected. PM<sub>1</sub> should largely exclude primary organic aerosol particles such as plant debris or resuspended dust, which are mostly found in larger particle size fractions. The HULIS or high molecular weight compounds studied here should be mainly associated with secondary organic aerosol mass or primary combustion particles.

Samples were collected on prebaked (during 8 h at 800 °C) quartz fiber filters (Schleicher and Schuell, Dassel, Germany) of 150 mm diameter using an appropriate sampling inlet (PM<sub>1</sub> impactor) on a high volume sampler (Model DA 80, Digitel, Hegnau-Volketswil, Switzerland) at a flow rate of 500 L/min. Filter sampling was performed without denuders. Although positive or negative sampling artifacts cannot be excluded, earlier studies showed that sampling artifacts do most likely not affect the HULIS concentration in WSOC significantly (10, 12). After sampling, filters were stored in a freezer at -20 °C until analysis. Total particle mass of the samples was determined by weighing the filters before and after sampling under defined conditions (22 °C, 50% relative humidity).

**Extraction of Aerosol Samples.** Because of the detection method used here, ELSD, only solvent and extraction media with relatively high vapor pressure can be used because the solvent has to be evaporated completely within the thermodenuder part of the ELSD. Thus, the organic aerosol is extracted with ultrapure water (18 M $\Omega$  cm specific resistance) obtained with a nanopure reagent-grade water system (Barnstead, Basel, Switzerland) (i.e., only the water-soluble HULIS fraction is quantified in this study). This fraction possibly influences climate relevant particle properties such as hygroscopicity or cloud formation potential. In the literature, HULIS are also often extracted with alkali solutions similar to standard procedures for the extraction of humic and fulvic acids from soils (8, 15, 21).

Filters were cut into circular pieces of 5 mm diameter and extracted in water for 45 min in an ultrasonication bath. Then, the supernatant was filtered with 0.45  $\mu\text{m}$  pore size PVDF filters (Whatman, Clifton, NJ, and the filtered aqueous aerosol extracts were immediately used for the further analysis steps.

**Instrumentation.** Aqueous size-exclusion chromatography (SEC) measurements were performed with a system consisting of a six-port Rheodyne 7725i sample injection valve, a Shimadzu System Controller (SCL-10Avp), a Solvent Pump Module (LC-10ATvp), and a Membrane Degasser (DGU-14A). The SEC column used was an Ultrahydrogel 120 column (300 mm  $\times$  7.8 mm, dp 6  $\mu\text{m}$ , pore size 120 Å) with a hydroxylated polymethacrylate stationary phase (Waters, Ruppertswil, Switzerland). For all measurements, the eluted compounds were simultaneously detected by a Shimadzu dual lamp photodiode array detector (SPD-M10Avp, 190–800 nm) and an evaporative light-scattering detector (PL-ELS 2100, Polymer Labs, Darmstadt, Germany). Data acquisition and processing were performed by VP-Class 5.0 chromatographic software. As an eluent, ultrapure water (18 M $\Omega$  cm) solutions with volatile additives were used. For the most sensitive measurements (i.e., low background signal levels), only volatile additives that evaporate completely in the thermodenuder of the ELSD should be used, such as ammonium acetate that was used in our study.

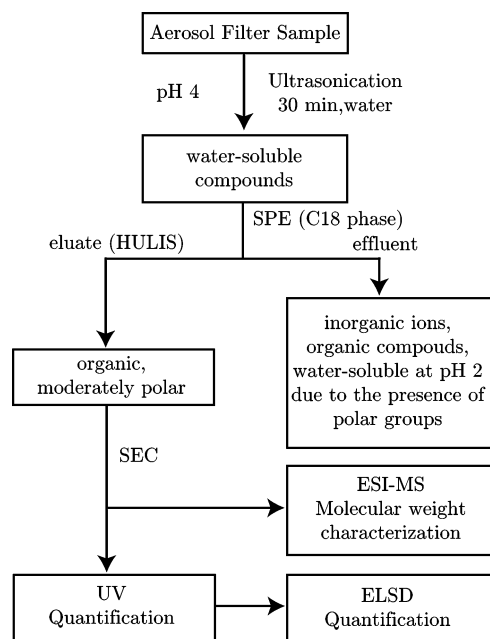
For qualitative comparisons, particle extracts were also analyzed with electrospray ionization mass spectrometry (ESI-MS) using a quadrupole time-of-flight MS (Q-ToF Ultima, Waters/Micromass, Manchester, UK) performed in negative ionization mode with the following settings: cone voltage 80 V, capillary voltage -2.5 kV, source temperature 125 °C, desolvation temperature 250 °C. The software MassLynx V4.0 was used for data acquisition.

All filter samples were also analyzed for organic and elemental carbon (OC and EC) using the thermal optical transmittance method (TOT) (26). The filter analyses were done with a semi-continuous OC/EC analyzer (Sunset Laboratory Inc., Tigard, OR) operated offline with a laboratory instrument using the temperature profile according to the NIOSH protocol (32).

## Results and Discussion

Before quantification, HULIS had to be separated from other water-soluble compounds in the filter extracts. This was done in two steps, as shown schematically in Figure 1. First, inorganic ions were removed with a C-18 solid-phase extraction (SPE) column in the effluent. Second, low molecular weight and volatile compounds remaining in the eluate fraction of the SPE were separated from HULIS with SEC and ELSD. The effectiveness of these separation steps was followed with ELSD and UV (step 1) and ESI-MS (step 2). Experiments were carried out to verify the optimum set of parameters for the aqueous SEC, which are important for the size separation as well as for the ELSD detection, as described in detail next.

**Separation of Inorganic Ions from HULIS.** Because inorganic ions such as sulfate or nitrate cause a signal in the ELSD, they have to be separated from HULIS prior to detection. Because of secondary effects on the SEC column (e.g., by electrostatic interaction between analyte and stationary phase), it was not possible to separate sulfate from HULIS on a SEC column, in contrast to other inorganic ions such as nitrate. Several different modifiers were tested at various pH values, such as trifluoroacetic acid, formic acid, acetic acid, ammonium acetate, ammonium carbonate, and triethylamine, at concentrations ranging from 0.01 to 0.1 M and with different columns (Ultrahydrogel 120, Waters; Biobasic SEC 60, Thermo; RP-18 LiChrospher, Merck, Whitehouse Station, NJ). However, it was not possible to success-



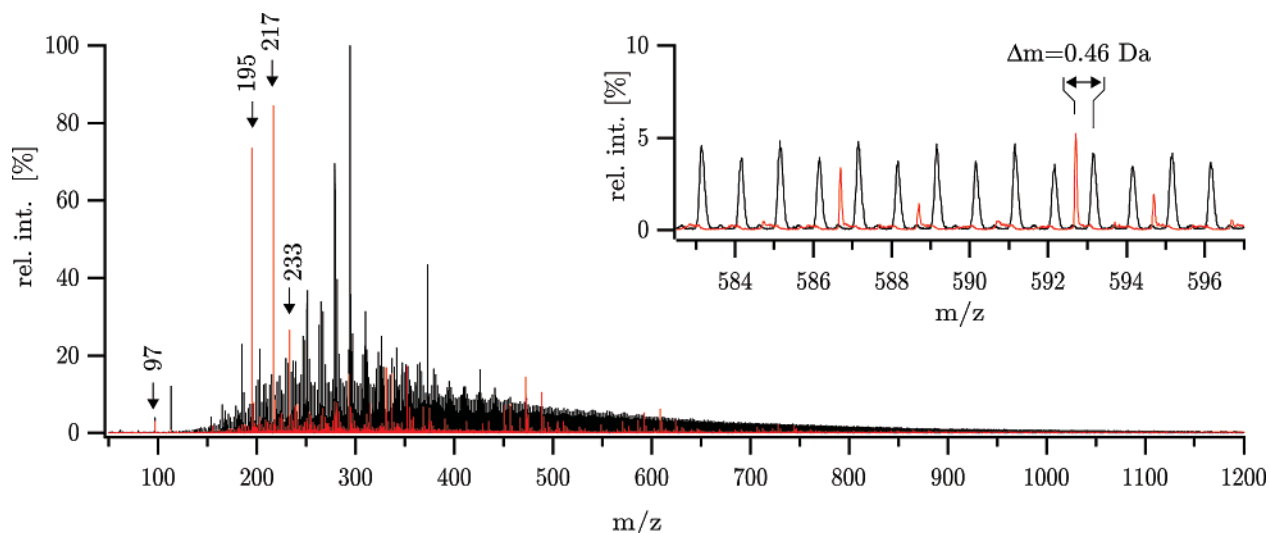
**FIGURE 1. Schematic of the treatment steps in the HULIS analysis.** After extraction of the filter samples, the two main separation steps are solid-phase extraction (SPE) and size-exclusion chromatography (SEC). The ELSD detector also separates HULIS from volatile, small organic compounds in its thermodeuder (evaporator tube) unit.

fully separate sulfate from the HULIS retention time window. A selective precipitation of sulfate was tested by adding barium hydroxide to a standard mixture of Suwanne River fulvic acid (SRFA, International Humic Substance Society) and sulfate. Although sulfate could be successfully removed, parts of the SRFA were also precipitated due to the required excess of barium. Solid-phase extraction (SPE), a method introduced and developed by Varga et al. (16) to isolate water-soluble HULIS from inorganic ions, was shown to be successful. This method was adapted to meet our experimental conditions. SPE DSC-18 columns (Supelco, Bellefonte, PA) were cleaned prior to usage with water and methanol. A total of 0.65 mL of an aqueous HULIS sample extract, acidified with 20  $\mu$ L of 0.1 M trifluoroacetic acid to about a pH of 2, was passed through the C-18 column. The column was then rinsed with 0.3 mL of acidified water (pH 2), and finally, the retained organic compounds, the HULIS fraction,

were eluted from the column with 0.5 mL of methanol. The effluent (waste fraction) as well as the eluate (HULIS fraction) were evaporated to dryness in a rotational vacuum concentrator (RVC 2--18, Christ, Osterode, Germany) at 50  $^{\circ}$ C and redissolved in a known amount of pure water depending on the sample concentration. ELSD measurements of standards (SRFA) confirmed that sulfate was successfully separated from HULIS and found in the effluent only. The overall mass balance for the SPE procedure of effluent and eluate accounts for  $86 \pm 12\%$  ( $n = 7$ ) of the total mass according to ELSD measurements for the measured ambient samples.

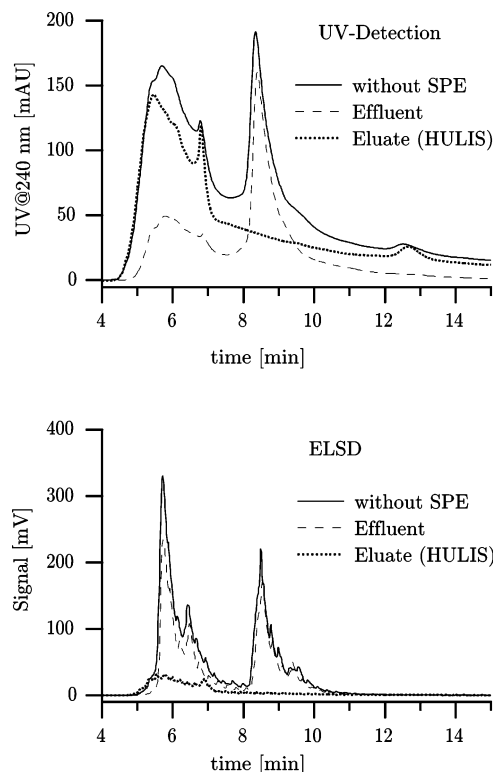
**Molecular Weight Distribution of SPE Fractions Determined by ESI-MS.** The two SPE fractions were analyzed by ESI-MS to determine the molecular weight distribution of the compounds in these two fractions (see Figure 2). Comparing the effluent (waste fraction) and eluate (HULIS fraction), it is evident that most of the higher molecular weight compounds are found in the eluate. The most intense peaks in the eluate were found in the range of  $m/z$  200–500 with highest masses up to  $m/z$  800. In the effluent fraction, most peaks were found below  $m/z$  300 with dominant peaks at  $m/z$  97, 195, 217, and 233. Only a few isolated peaks were measured above  $m/z$  300 (i.e., in the high molecular weight range). Test experiments with pure sulfate showed that the sulfate monomer at  $m/z$  97 ( $\text{HSO}_4^-$ ) gives only a weak signal, but dominant peaks were measured for the deprotonated dimer ( $\text{H}_2\text{SO}_4 \cdot \text{HSO}_4^-$ ) at  $m/z$  195 as well as the potassium and sodium adducts of the dimer with an additional loss of a proton at  $m/z$  217 and 233, respectively. These ESI-MS analyses confirm that the sulfate remains entirely in the effluent fraction of the SPE (the sulfate peaks are indicated by arrows in Figure 2) and that the vast majority of the higher molecular weight compounds is found in the eluate fraction.

An expanded view of the mass region between  $m/z$  583–597 is shown in an insert in Figure 2. The effluent shows peaks that have a mass deficiency of about  $\Delta m = 0.46$  Da as compared to the compounds in the eluate. This difference slightly increases with molecular weight. This consistent mass difference for the two SPE fractions can be explained with highly oxidized compounds present in the effluent.  $^{16}\text{O}$  has an exact mass of 15.9949 Da, which is lower than its nominal mass (i.e., 16 Da), but other elements (e.g., H) in contrast have a slightly higher exact mass (e.g., 1.0078) than their respective nominal masses. Thus, a highly oxidized compound has a lower exact mass than a less oxidized compound with the same nominal mass. During SPE separation with a C-18 phase, it can be expected that very polar compounds



**FIGURE 2. ESI-MS in negative mode for both the (red line) effluent and the (black line) eluate of the SPE separation step.** The inset shows the mass difference due to the different polarity (C/O ratio) of compounds in these two fractions.





**FIGURE 3.** Chromatograms of water-soluble aerosol components before and after SPE separation using ELSD and UV (at 240 nm) as detection methods.

are not retained on the column (i.e., ending up in the effluent fraction). However, as the ESI-MS result also show, these compounds have mostly masses below  $m/z$  300.

**SEC Separation.** After SPE separation of inorganic ions and small organic compounds, the filter extracts were separated with a SEC column coupled on-line to ELSD. Typical SEC chromatograms of both UV and ELSD detection of an ambient sample are shown in Figure 3.

Optimum ELSD working conditions for a SEC mobile phase of 0.1 M ammonium acetate in water were found using a sheath gas flow of 1.25 L/min and nebulizer and evaporation tube temperatures of 60 and 90 °C, respectively. Linear calibration was obtained using an SRFA standard with concentrations of 0.1, 0.4, 1, and 2 mg/mL.

Comparing the ELSD signal of the eluate and the effluent of the SPE with the chromatogram of the sample before the SPE (see Figure 3) shows the efficient separation of inorganic salts, in particular, the sulfate at 5.9 min and nitrate at 8.5 min from the HULIS fraction. The high molecular weight compounds (eluate) elute early in the chromatogram (5.0–7.5 min retention time). Possibly, some aromatic compounds elute very early (before 5.0 min retention time) as seen in the early increase of the UV signal, when no ELSD signal is measured yet.

The ELSD chromatogram (Figure 3) of the eluate (HULIS) fraction shows only poorly resolved peaks between 5.0 and 7.5 min retention time. In an earlier study (10), we used polymethacrylic acid (PMA) and dicarboxylic acids for size calibration of the SEC column, which showed that compounds eluting earlier than 5.5 min with our present setup have a molecular weight of  $>250$  Da. Among the commercially available water-soluble polymers for size calibration, PMA was shown to be chemically most similar to HULIS (10). However, in this study, on-line ESI-MS measurements of the eluate (HULIS) fraction, separated with the SEC column, showed no significant differences in the mass spectra throughout a retention time of 5.0–7.5 min, where peaks

**TABLE 1.** List of Low Molecular Weight Compounds That Were Separated from HULIS Either during the SPE Step or in the Thermodenuder Part of the ELSD<sup>a</sup>

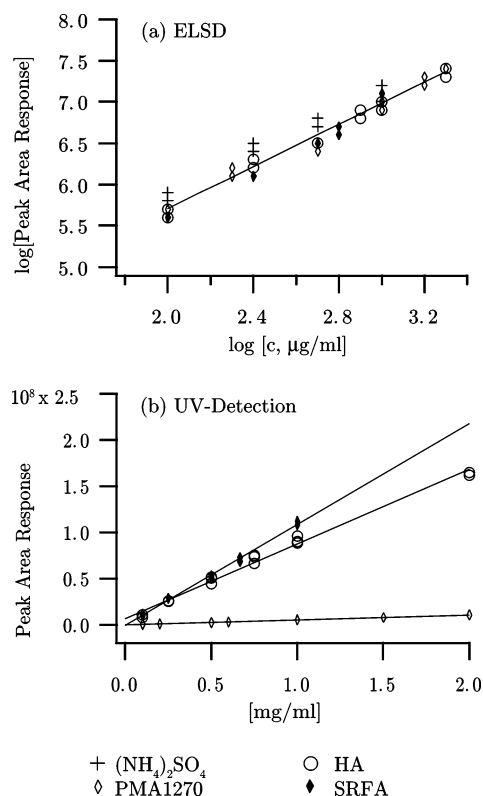
compound	separated from HULIS by	
	SPE	evaporation in ELSD
C1–C6 monocarboxylic acids		*
oxalic acid	*	
succinic acid		*
malonic acid	*	
glutamic acid		*
adipic acid		*
D,L-malic acid	*	
4-hydroxybenzoic acid		*
citric acid	*	
D,L-lactic acid		*
Phenol		*
trifluoroacetic acid		*
ammonium nitrate	*	
ammonium sulfate	*	

<sup>a</sup> An asterisk indicates which compound is separated from HULIS during which step.

were detected with ELSD. Similar molecular weight distributions were measured throughout this retention time window. It has to be concluded that the compound mixture present in the eluate of the SPE separation is too complex to achieve a further size separation with one-dimensional SEC. Therefore, SEC was not successful to further purify the HULIS fraction from low molecular weight compounds.

However, the ELSD itself offers the possibility to separate high volatility compounds from low volatility compounds. In the thermodenuder part of the ELSD detector, small volatile compounds evaporate before the actual detection, whereas compounds with lower volatility (and higher molecular weight) were detected. At our working conditions, many known oxidized low molecular weight compounds, which have been identified in aerosols, evaporate in the ELSD, adding an additional separation dimension to the total separation scheme. Table 1 lists 19 water-soluble compounds that could be separated from the HULIS fraction either by ELSD or by SPE. Many less oxidized compounds (e.g., monocarboxylic acids) were not separated from HULIS by SPE as expected but were separated in the ELSD from HULIS because they evaporate in the thermodenuder part of the detector. Phthalic acid was the only compound tested that cannot be separated from HULIS with our methodology. For these reasons, all peaks measured with ELSD in the eluate (HULIS) fraction (retention time 5.0–7.0 min) were defined and quantified as HULIS.

**Quantitative Calibration of ELSD.** Four compounds were used to establish a calibration curve for the ELSD: ammonium sulfate, polymethacrylic acid with an average molecular weight of 1270 Da (PMA 1270), humic acid (HA, Fluka, Buchs, Switzerland), and SRFA of different concentrations were measured as shown in Figure 4. In Figure 4a, the ELSD calibration curves are shown. The peak area ( $A$ ) is related to the sample mass  $m$  by the following relationship  $A = am^b$ , where  $a$  and  $b$  are coefficients depending on the chromatographic conditions (28, 31). In a log–log plot, this translates into a linear calibration curve with slope  $b$  and intercept  $\log a$ , according to  $\log(A) = \log(a) + b \log(m)$ . All four calibration compounds result in one single linear calibration curve regardless of their chemical or optical properties (see Figure 4a). For comparison, Figure 4b shows the linear calibration curves for PMA1270, HA, and SRFA as measured with UV at 240 nm. Ammonium sulfate was not detected due to the lack of UV absorbance. The large differences in the slope of the calibration curves are due to the different UV absorption properties of the analytes. This



**FIGURE 4.** Comparison of ELSD (a) and UV (b) calibration (at 240 nm) curves for different organic and inorganic compounds. For ELSD, one single calibration curve is sufficient for all four compounds, whereas UV shows strong differences in the detector responses between the different calibration compounds.

comparison between UV and ELSD detection drastically shows the advantage of ELSD for the quantification of compounds with an unknown chemical structure. While concentration uncertainties with UV can easily exceed a factor of 30 (see Figure 4b), the uncertainty for ELSD is lower than a factor of 3. Modeling of the factors, which can influence the response behavior of the ELSD (e.g., the refractive index or density of the analyte), show that the sensitivity can vary by a factor of 2 as these properties change (33). The limit of detection of the ELSD was 0.01 mg/mL for SRFA. The relative standard deviation of ELSD measurements in this configuration for ammonium sulfate is in the range of 24% ( $n = 8$ ).

As described previously, the HULIS quantification always depends significantly on the separation procedures applied. Limbeck et al. (7) and Varga et al. (16) used similar separation procedures, where the main separation step is a solid-phase extraction step, similar to the first separation step in this study. Limbeck et al. (7) used in addition as second dimension a small ion exchange column before OC detection. Facchini et al. (23) and Decesari et al. (18) used a different separation approach with ion exchange separation. These methods define HULIS as water-soluble organic polyacids, whereas our method most likely includes also other high molecular weight compound classes. Other authors (e.g., ref 14) used electrophoretic separation to isolate HULIS. Without a side-to-side comparison, it is difficult to determine the effects of the different procedures on the quantification of HULIS.

**Ambient Aerosol Samples.** In Table 2, the data of a campaign in winter 2005–2006 is shown. Six weekly samples were collected with week 5 being split into two samples, 5a (4.7 days) and 5b (2.3 days). Samples 4 and 5a were collected during an extremely high PM1 period that was followed by a period with low PM1 due to strong snowfall (sample 5b).

**TABLE 2.** Weekly PM1 Winter Samples Collected at a Suburban Site in Dübendorf, Switzerland, between December 15, 2005 and January 26, 2006<sup>a</sup>

parameter	1	2	3	4	5a	5b	6	Ø
PM1 ( $\mu\text{g}/\text{m}^3$ )	12.2	17.7	15.6	28.8	42.0	9.0	22.8	21.2
UV ( $\mu\text{g}/\text{m}^3$ )	0.41	0.56	0.72	1.16	1.73	0.21	1.12	0.84
UV/PM1 (%)	3.4	3.2	4.6	4.0	4.1	2.3	4.9	3.8
ELSD ( $\mu\text{g}/\text{m}^3$ )	0.46	0.70	0.78	1.40	2.29	0.51	1.27	1.06
ELSD/PM1 (%)	3.8	3.9	5.0	4.9	5.5	5.6	5.5	4.9
ELSD/UV ratio	1.1	1.3	1.1	1.2	1.3	2.5	1.1	1.4
OM ( $\mu\text{g}/\text{m}^3$ )	3.7	7.2	6.8	11.2	13.0	1.4	9.0	7.5
ELSD/OM (%)	12.5	9.7	11.5	12.5	17.6	35.2	14.1	16.2

<sup>a</sup> Exceptions are samples 5a and 5b, which correspond to 1 week divided into 5 and 2 days, respectively. A comparison of the HULIS concentrations quantified with UV and ELSD, respectively, shows that the UV quantification underestimates the HULIS concentration up to a factor of 2.5 as compared to ELSD.

The high PM period was due to a meteorological situation often found in the winter in the Swiss lowlands, the build-up of a stable inversion layer, accumulating air pollutants in a shallow boundary layer. Sample 6 was extracted and measured 3 times with ELSD resulting in a relative standard deviation of 13% for the HULIS quantification.

The water-soluble HULIS concentration determined with ELSD during this winter period was in the range of 0.46–2.29  $\mu\text{g}/\text{m}^3$ . On average, HULIS make up 4.9% of PM1. The HULIS fraction of PM1 seems to be quite constant over time. After a snowfall when only fresh particles should be present (sample 5b), there was no change of the HULIS to PM1 ratio as compared to aged particles during the inversion period (samples 4 and 5a). This suggests that HULIS are generated rather fast in the atmosphere or are emitted directly (e.g., by wood burning). The samples were quantified in parallel by UV at a wavelength of 240 nm using SRFA as a calibration standard. The ratio of ELSD/UV varies substantially from 1.1 to 2.5, showing that the optical properties of HULIS can change significantly with time or meteorological conditions. This emphasizes that optical methods should be used with care for HULIS quantification. This result is in agreement with earlier studies comparing UV quantification with OC analysis of HULIS, showing that UV underestimates the HULIS concentration by a factor of about 2 (7).

OC was measured 3 times per filter (except for filters 5a and 6, which were measured twice) with relative standard deviations in the range of 5%. The organic carbon (OC) was converted to organic matter (OM) using a factor of 1.9 (34). OM concentrations in the range of 1.4–13  $\mu\text{g}/\text{m}^3$  were determined (see Table 2), which is on average 33% of the PM1 mass. HULIS on average make up 16.2% of the OM. Once again, sample 5b differs remarkably from the other samples. The percentage of HULIS in OM in sample 5b is roughly doubled and accounts for 35.2%. This shows again that the HULIS fraction is quickly formed in (or directly emitted into) the atmosphere and that HULIS are chemically different after precipitation. Limbeck et al. (7), for example, used a similar methodology to ours (C-18 SPE and ion chromatographic separation). They determined the water-soluble HULIS carbon average percentage of OC to 12.1%, which is comparable to our results. Other authors using different separation methods determined 15–60% HULIS in WSOC (8, 14, 18, 22, 23).

The ELSD technique for HULIS quantification was shown to be successful. This detection method allows the quantification of structurally unknown compounds such as HULIS. SEC appears to be insufficient to further separate HULIS into fractions of different molecular weights. However, combining the power of SPE to exclude inorganic ions and ELSD, which discriminates HULIS against many low mo-

lecular weight compounds in its thermodenuder part due to evaporation, allows us to obtain accurate HULIS concentrations. HULIS concentrations have values in the low  $\mu\text{g}/\text{m}^3$  range and make up about 10–35% of the whole organic PM1 mass depending on meteorological conditions. The molecular mass distribution for the quantified HULIS is in the range of 200–500 Da.

## Acknowledgments

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