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Application of Non-Ionic Solid Sorbents (XAD Resins) for the Isolation and Fractionation of Water-Soluble Organic Compounds from Atmospheric Aerosols

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Abstract. A detailed procedure using non-ionic macropourous XAD-8 and XAD-4 resins is presented for the isolation and fractionation of aerosol water-soluble organic compounds (WSOC) from aerosol samples. The procedure entails adsorption of WSOC fraction onto XAD-8 and XAD-4 resins, desalting of the adsorbed organic material with ultra-pure water, elution of the retained organic matter with 40% MeOH solution and freeze-drying. Due to resin's different properties and to certain hydrophobic/hydrophilic interactions between the resin polymers and the organic matter, two major fractions were obtained; namely the XAD-8 and the XAD-4 eluates. The XAD-8 eluate, which accounts for 55–60% of total aerosol WSOC, is represented by partially acidic compounds with significant hydrophobic moieties. The XAD-4 fraction holds few conjugated systems and a higher content of hydrophilic structures with low molecular size, and accounts for 9% of total WSOC. The isolated WSOC sub-fractions were nearly free from inorganic species, and successful recoveries of organic matter from the resins were accomplished. With this procedure the XAD-8 eluate yields a mixture representative of those WSOC that are highly conjugated compounds in atmospheric aerosols. It also allows a successful characterisation of the organic material by advanced analytical techniques without the interference of inorganic species present in the original sample of atmospheric particles.

Key words: XAD resins, isolation, fractionation, water-soluble organic compounds, atmospheric aerosols

1. Introduction

Currently, there is a consensus opinion about the importance of water-soluble organic compounds (WSOC) of aerosol particles in different atmospheric processes. Some investigators have estimated that this fraction constitutes a substantial part (∼20–70%) of particulate organics in the atmosphere (Zapolli *et al.*, 1999; Decesari *et al.*, 2000; Facchini *et al.*, 1999). In what concerns the chemical composition of this water-soluble organic fraction only a small percentage is known. A theoretical study conducted by Saxena and Hildemann (1996) provided a list of candidate multifunctional compounds (e.g. diacids, polyols, amino acids) that may contribute to the WSOC fraction of atmospheric aerosol. Facchini *et al.* (1999) identified about 120 individual compounds in a polluted area, including aliphatic dicarboxilic acids, sugars, aliphatic alcohols and aliphatic carboxylic acids. However, these compounds only accounted for less than 5% of the total WSOC. Recent studies (Havers *et al.*, 1998; Decesari *et al.*, 2000; Gelencsér *et al.*, 2000; Krivácsy *et al.*, 2000, 2001; Kiss *et al.*, 2002) have anticipated that a considerable amount of the WSOC might be of high molecular weight presenting physical and chemical properties similar to those of aquatic humic substances. Additionally, this type of organic matter has been found in both urban (Havers *et al.*, 1998; Zappoli *et al.*, 1999) and rural locations (Krivácsy *et al.*, 2001; Kiss *et al.*, 2002) suggesting that they are ubiquitous constituents of atmospheric aerosols.

The presence of these so-called humic-like substances in atmospheric aerosols has prompted the development of new analytical methodologies based on fractionation schemes of aerosol WSOC (by preparative chromatography on XAD-2 resin (Andracchio *et al.*, 2002), size-exclusion chromatography (Kricácsy *et al.*, 2000; Andracchio *et al.*, 2002), ion-exchange chromatography (Decesari *et al.*, 2000) and solid-phase extraction (Varga *et al.*, 2001)) followed by functional group analysis (e.g. Proton Nuclear Magnetic Resonance and Infrared Spectroscopies).

Regardless the method applied into the characterisation of aerosol WSOC fraction, it is clear that the study of its chemical and environmental significance is greatly dependent on the limitations imposed by both the extraction and isolation procedures. The WSOC present in the aqueous extract of aerosol particle interact with other dissolved species, both organic and inorganic. Before their chemical and physical properties can be defined thoroughly, the WSOC fraction must be isolated from other compounds, especially from inorganic species since they interfere with the application of advanced analytical techniques such us Proton Nuclear Magnetic Resonance (^{1}H NMR), Solid State ^{13}C Nuclear Magnetic Resonance (^{13}C NMR) or Fourier Transform Infrared (FTIR) Spectroscopies. Simultaneously, it is of great advantage for the laboratory study of WSOC fraction if the isolation procedure produces unaltered material and ensures a representative mixture of the WSOC from the original atmospheric aerosol.

Taking into account the above mentioned requirements, this work presents a new methodology for the isolation of WSOC material from aqueous extracts of fine aerosols particles. In this method non-ionic solid sorbents (XAD resins, Amberlite) were applied into the isolation and fractionation of the WSOC fraction. Dissolved Organic Carbon (DOC) measurements and UV absorption at 250 nm were used in order to carry out a complete assessment of organic matter retention, recovery and losses in the resins. Several recovery tests on the resins were also performed in order to evaluate possible bleeding problems of the resin polymers during the sample handling.

2. Fundamentals

XAD resins are non-ionic macropourous copolymers with a large surface area. The exact mechanism for the organic solutes adsorption onto these resins is not fully understood, but it appears to be related to the molecular size of the solute and to certain hydrophobic/hydrophilic interactions between the organic solutes and the sorbing solid under pre-adjust conditions. Due to its high adsorptive capacity, XAD-8 resin has been widely used for the isolation of humic substances from aquatic environments (Malcolm, 1991; Burba *et al.*, 1995, Boerschke *et al.*, 1996; Esteves *et al.*, 1995; Peuravuori *et al.*, 1997; Santos and Duarte, 1998). Briefly, the isolation procedure is carried out by adsorption of the dissolved organic matter on a XAD-8 column at pH 2, followed by elution with a sodium hydroxide solution. In order to prevent organic matter oxidation at alkaline pH, the eluted organic matter is passed through a cationic exchanger column (in the H^+ form) or, alternatively, acidified to pH 4–5 with a hydrochloric acid solution. The humic substances isolated by this procedure are further separated into fulvic acids and humic acids and desalted by a procedure described elsewhere (Malcolm, 1991).

Traditionally, the research of the chemical and physical properties of humic substances from freshwaters only takes into account the compounds extracted with the XAD-8 resin. The compounds that are not retained onto this resin are usually discarded. With the purpose of characterising the organic fraction missed by XAD-8 resins, tandem XAD-8/XAD-4 resins have been employed into the isolation of dissolved organic matter from natural waters (Boerschke *et al.*, 1996; Santos and Duarte, 1998; Santos *et al.*, 2001). Those resins differ in pore size, surface area, polarity and composition as shown in Table I. Hence, by using the XAD-8 and XAD-4 resins connected in series, the most hydrophobic macromolecular organic solutes are retained in the macropoures of the XAD-8 resin while the most hydrophilic and of low molecular sizes are retained in the XAD-4 resin. It also becomes obvious that in this procedure, besides isolation and concentration of the dissolved organic matter, a fractionation of the organic matter is also accomplished according to the molecular size of the solutes and to certain hydrophobic/hydrophilic interactions between the resin polymers and the organic matter.

Table I. Properties of the resins used in the isolation procedure of the dissolved organic matter (reproduced from Malcolm (1991))

XAD resin	Surface area (m^2/g)	Pore size (Å)	Polarity	Composition
XAD-8	250	250	Slight	Acrylic esther
XAD-4	750	50	None	Styrene-divinylbenzene

3. Materials and Methods

3.1. AEROSOL SAMPLING

High-volume aerosol samples were collected in Aveiro (Portugal) in two different sampling stations. One aerosol sample was collected 10 Km from the coastal zone ($40°38'$N, $8°39'$W) and has influences from both urban and oceanic environments. Four aerosol samples were collected in a rural location with high agricultural activity ($40°57'$N, $8°64'$W) during the summer and autumn seasons during one-month period (Duarte *et al.*, 2004). The aerosol samples were collected on a pre-fired (at 500 °C) quartz fibre filters (20.3 × 25.4 cm; Whatman QM-A) for sampling periods of 7 days with a air flow rate of 1.13 m^3 min^{-1}. High volume sampling is necessary in order to collect enough material for future detailed chemical characterisation. The high-volume collector was provided with a pre-separator for excluding particles larger than 2.5 μm. This size-cut was accomplished using a Sierra PM_{10} inlet in series with a Sierra high-volume impactor and a back-up filter in front of the quartz filter.

The total mass concentration of the collected particulate matter in each filter was determined by weighting the filter under controlled moisture conditions before and after sampling. After this procedure the filters were wrapped with aluminium paper and stored frozen until further analysis. The concentration of total carbon (TC) and elemental carbon (EC) was measured for the collected aerosols by means of a thermo-optical method (Pio *et al.*, 1994).

3.2. EXTRACTION OF THE AEROSOL SAMPLES

One portion of the filter collected in the first sampling station was extracted with 50 mL of ultra-pure water by mechanical agitation during 5 min. The final slurry so obtained was filtered through a pre-fired quartz filter. The aqueous extract was further filtered through a syringe filter (PTFE, Gelman Sciences) of 0.22 μm pore size. The final slurry was additionally subjected to another run of the extraction procedure. This second aqueous extract was kept and treated separately from the first aqueous extract.

The filters collected in the second sampling station were extracted with 150 mL of ultra-pure water by mechanic stirring during 5 min plus ultrasonic bath during 15 min. The final slurry was filtered through a membrane filter (PVDF, Gelman Sciences) of 0.22 μm pore size (Duarte *et al.*, 2004).

3.3. XAD SORBENTS

Monomers and impurities of Amberlite (Supelco) XAD-8 and XAD-4 resins were removed by hot Soxhlet extraction with a series of solvents of analytical grade: methanol, acetonitrile and diethyl ether. After this cleaning sequence, the resins were cleaned once more with methanol and then stored in methanol before use.

Two glass columns containing the XAD-8 and XAD-4 resins (15 mL) were set up for the isolation and fractionation of the WSOC of the aerosol. Prior to usage, resin columns were rinsed with approximately 100 column volumes of ultra-pure water to completely free the resins of methanol. After this procedure, both resins were rinsed with one column volume of a sodium hydroxide solution (0.1 M) and then rinsed with one column volume of a hydrochloric acid solution (0.1 M). As the sample pH will be lowered to pH $\approx$ 2.0, the resins were finally rinsed with one column volume of 0.01 M HCl.

3.4. ISOLATION AND FRACTIONATION OF AEROSOL WSOC

Figure 1 presents the scheme of the samples preparation procedure. The aqueous samples were first acidified to pH = 2.2 ($\pm$0.2) before being pumped onto the XAD-8 resin at a rate of 0.8 mL/min. During this first concentration stage the organic matter is retained on the top of the XAD-8 column yielding a yellowish brown colour to the resin. The down flow effluent from the XAD-8 resin was firstly collected in a reservoir before being pumped onto the XAD-4 resin at the same flow rate. After this two concentration stages the organic matter adsorbed onto the resins was down washed with one column volume of ultra-pure water, at a rate of 0.8 mL/min, to remove the inorganic species. The desalting effluents from XAD-8 and XAD-4 resins were also collected separately. The organic matter retained in both resins was then back eluted with a solution of methanol/ultra-pure water in the proportion 2:3 (40% MeOH). The volumes of the 40% MeOH solution used

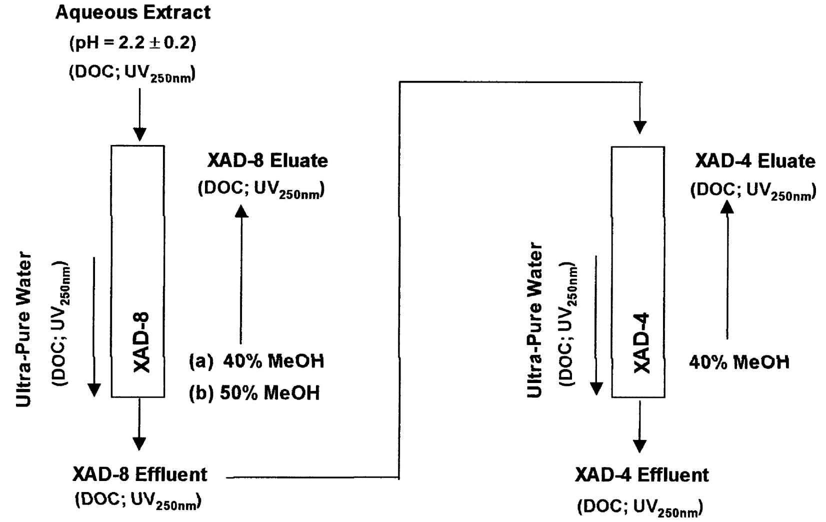

Figure 1. Scheme of the tandem XAD8/XAD-4 isolation and fractionation procedure of the aerosol WSOC ((a) 40% MeOH and (b) 50% MeOH refers to the sequential elution procedure of the XAD-8 resin with solutions of ultra-pure water/methanol in different proportions).

in XAD-8 and XAD-4 columns were, respectively, 80 mL and 50 mL. In the case of the XAD-8 resin, after elution a light yellowish band still remained on the top of the column. In order to improve the recovery of the retained organic matter a second elution step was carried out with a solution of methanol/ultrapure water in the proportion 1:1 (50% MeOH). All eluates were evaporated almost to dryness in a rotary evaporator at 30 °C and then re-dissolved in ultra-pure water. Finally, the eluates were freeze-dried and kept on a dessicator over silica gel for further analysis. Before and after each step, the DOC content and the UV-VIS spectra of the influents and effluents from the XAD columns and also of the desalting effluents and reconstituted eluates were measured for assessing losses of organic matter during the extraction procedure.

Owing to the small amounts of water-soluble organic matter usually extracted from atmospheric particles, the WSOC fractions extracted from the aerosol samples collected in the rural location in each sampling season were combined prior to their isolation by the XAD resins.

3.5. DISSOLVED ORGANIC CARBON (DOC) ANALYSIS

The DOC content of the samples was measured by an automated colorimetric method (Wangersky, 1993). The sample is acidified and purged with nitrogen to remove the inorganic carbon. A buffered (borate) persulfate solution is added to the acidified sample and the resulting solution is irradiated in an UV reactor. After that, a hydroxylamine solution is added to oxidize dissolved organic matter. The resulting solution enters into a dialyser where the previously generated carbon dioxide diffuses through a gas permeable silicone membrane into a weakly buffered phenolphthalein indicator solution. The absorbed carbon dioxide causes a decrease in phenolphthalein colour intensity due to changes in pH. The intensity of colour change, being proportional to the mass of diffused carbon dioxide, is measured in a flow cell at 550 nm.

3.6. UV-VIS SPECTROSCOPY

The spectra of the samples were recorded on a UV-VIS spectrophotometer Shimadzu Model UV 2101 PC in 1 cm path length quartz cells. The ultra-pure (Milli-Q) water was used as a blank.

4. Results and Discussion

4.1. TOTAL CARBON BALANCE OF ATMOSPHERIC AEROSOLS

The concentration of total carbon (TC), water-soluble organic carbon (WSOC) and elemental carbon (EC) was measured for each aerosol sample. The organic carbon (OC) content was calculated as the difference TC – EC and the results are shown

Table II. Concentrations of total aerosol mass, total carbon (TC), elemental carbon (EC), organic carbon (OC) and water-soluble organic carbon (WSOC) in the collected samples (SD of three measurements in brackets)

Sample	Total fine aerosol (μg m^{-3})	TC (μgC m^{-3})	OC (μgC m^{-3})	EC (μgC m^{-3})	WSOC (μgC m^{-3})
Rural-summer					
S1	24.9	3.0 (±0.2)	2.5 (±0.2)	0.5 (±0.01)	1.2 (±0.1)
S2	20.6	3.0 (±0.2)	2.4 (±0.2)	0.6 (±0.01)	1.0 (±0.1)
S3	21.6	3.1 (±0.1)	2.5 (±0.1)	0.5 (±0.2)	1.2 (±0.04)
S4	20.1	3.1 (±0.5)	2.4 (±0.4)	0.7 (±0.2)	1.0 (±0.01)
Rural-autumn					
A1	30.9	11.4 (±0.9)	10.0 (±0.7)	1.4 (±0.2)	3.0 (±0.1)
A2	16.1	5.7 (±0.6)	4.4 (±0.5)	1.3 (±0.1)	1.8 (±0.1)
A3	16.8	6.2 (±0.2)	4.9 (±0.3)	1.2 (±0.1)	1.8 (±0.1)
A4	18.3	5.4 (±0.3)	4.4 (±0.1)	1.0 (±0.1)	2.0 (±0.1)
Urban/oceanic	28.5	9.0 (±0.3)	8.3 (±0.5)	0.6 (±0.3)	5.4 (±0.1)

in Table II. It can be observed that the OC contributes to more than 80% of the TC content of the aerosol samples. The EC constitutes 7% of the TC content for the urban/oceanic sample and 18–22% of TC content for the rural samples (Duarte *et al.*, 2004). It was also found that the most abundant organic carbon fraction is WSOC, which accounted for 60% of TC for the urban/oceanic sample and 31–36% of TC for the rural samples (Duarte *et al.*, 2004). These values are in agreement with those reported in the literature for atmospheric aerosols collected in areas characterized by intensive industrial, trading and agricultural activities (Decesari *et al.*, 2000) and also in areas influenced by both marine and continental air (Zappoli *et al.*, 1999). Additionally, for the aerosol samples collected in the rural location, it can also be observed a seasonal variation in the concentration of TC, OC and WSOC, with higher values during the autumn and lower values during the summer season (Duarte *et al.*, 2004).

4.2. ADSORPTION AND FRACTIONATION OF WSOC IN XAD RESINS

UV-VIS spectra were recorded for each WSOC fraction, XAD-8 and XAD-4 eluates and XAD-8 and XAD-4 effluents of each aerosol sample and they were found very similar. Figure 2 shows the UV-VIS spectra of the WSOC fraction, XAD-8 and XAD-4 eluates and XAD-8 and XAD-4 effluents of the sample collected in the urban/oceanic location. The spectra are similar to those of natural humic substances, decreasing monotonically with increasing wavelength. However, spectra from both XAD-8 and XAD-4 effluents and also from XAD-4 eluate show a shoulder in the

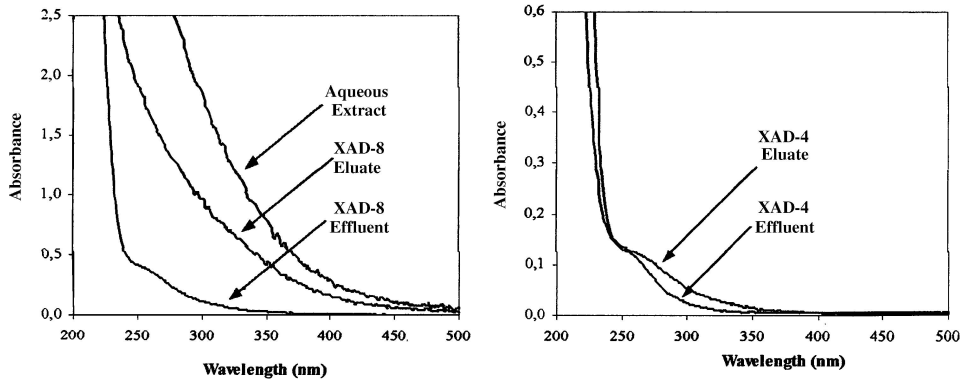

Figure 2. UV-VIS spectra of the aqueous extract (WSOC from the urban/oceanic aerosol sample), XAD-8 and XAD-4 eluates and XAD-8 and XAD-4 effluents.

region 250–300 nm. Absorbance in this UV region is usually assigned to π–π^* electron transitions of unsaturated systems (Williams and Fleming, 1973; Traina *et al.*, 1990; Chin *et al.*, 1994). These differences in the spectral features highlight the fractionation process imposed by the XAD-8 resin into the original WSOC fraction during the first concentration stage.

Table III reports an average distribution and percentage of recovery of DOC and UV absorbance at 250 nm (UV_{250}) of the isolation procedure of the WSOC samples. Seventy-six to 82% of the WSOC was retained in the XAD-8 resin, while 14–16% of the total WSOC fraction was retained in the XAD-4 resin. Only 4–9% of the WSOC passed through both XAD-8 and XAD-4 resins. In the case of UV absorption at 250 nm, 87–94% of the total UV absorption of the aqueous extract was retained in the XAD-8 resin, while only 5–9% of the total UV absorption was retained in the XAD-4 resin. Approximately 4% of the total UV absorption passed through both XAD columns.

During the desalting stage, with ultra-pure water, of the organic matter retained in the XAD-8 column it was observed a washout of colour from the column, which is a consequence of very weakly retained compounds that are desorbed together with the inorganics. It was found that 13–15% of the WSOC precluded the XAD-8 resin whereas 5–9% of the total UV_{250} was excluded. In the desalting procedure of the XAD-4 column, the loss of organic material and UV absorption was not higher than 5% and 3%, respectively.

The back elution of the retained organic material in the XAD-8 resin was not complete with 40% MeOH solution, and the subsequent elution with 50% MeOH solution only improved the organic matter recovery in approximately 3%. A similar value was obtained for UV absorption at 250 nm. Approximately 5–10% of the retained organic material was found irreversible adsorbed onto the resin polymers, causing its permanent discoloration. In the case of UV_{250}, 3–10% of the total UV absorption was found irreversibly adsorbed. These observations indicate a

Table III. Distribution and percentages of recoveries of WSOC in the fractions obtained from the isolation procedure

Fractions	Rural-summer DOC	Rural-summer $UV_{250\,nm}$	Rural-autumn DOC	Rural-autumn $UV_{250\,nm}$	Urban/oceanic DOC	Urban/oceanic $UV_{250\,nm}$
Retained in XAD-8 resin	75.7 (±2.2)	87.0 (±0.7)	75.9 (±3.8)	93.6 (±2.2)	82.1 (±0.2)	89.3 (±0.2)
Retained in XAD-4 resin	15.7 (±2.3)	9.2 (±1.1)	15.9 (±2.6)	5.2 (±2.1)	13.7 (±0.2)	7.2 (±0.1)
Not retained	8.8 (±1.5)	3.8 (±0.8)	7.9 (±2.3)	1.2 (±0.2)	4.3 (±0.1)	3.6 (±0.2)
Recovery from XAD-8 resin	55.2 (±2.9)	72.1 (±2.0)	54.1 (±7.1)	79.3 (±6.7)	62.0 (±2.0)	78.0 (±3.0)
Recovery from XAD-4 resin	9.7 (±1.6)	4.9 (±0.4)	9.3 (±2.0)	3.3 (±1.3)	9.3 (±0.1)	3.0 (±0.1)

Note. The table indicates the average value from the replicates (two for the urban/oceanic sample and ten for each rural sample (Duarte *et al.*, 2004)), and the standard deviation of the results is indicated in brackets.

very strong interaction between some highly hydrophobic moieties and the XAD-8 polymers and that some compounds become permanently occluded within the pores of the resin. In what concerns the XAD-4 resin, the irreversible adsorption of the organic matter and UV absorption onto the resin polymers was found negligible (not more than 1% and 2%, respectively).

In what concerns the organic matter recovered from the resins, 55–62% of the carbon was found in the XAD-8 eluate, while 72–79% of the total UV_{250} is achieved. For the XAD-4 fraction, approximately 9% of the retained organic material is eluted whereas only 3–5% of the total UV_{250} is recovered. These variations in percentage of recovery are probably due to the different composition of the aerosol samples. The low relative error for the XAD-8 and XAD-4 eluates from the replicates of each sample preparation procedure, less than 13% for both DOC and UV_{250} measurements, indicates the reliability of the developed methodology. However, for the XAD-4 eluate of the rural sample collected in the autumn season the relative error was found higher.

The results reported in this study were further compared with those recently developed by other authors (Andracchio *et al.*, 2002; Varga *et al.*, 2001), although they used different combinations of adsorbents and elution solvents. The principle of isolating the WSOC from the inorganic salts is common to all methods. However, the fractionation methods described in the literature were based on the hydrophobic and acidic character of the organic compounds. Specifically, the fractionation of WSOC samples is achieved by applying different solvents (Andracchio *et al.*, 2002) or by changing sample pH throughout the fractionation procedure (Varga *et al.*, 2001). Conversely, the method developed in the present study just takes into account the molecular size of the solutes and certain hydrophobic/hydrophilic interactions between the organic solutes and the adsorbing solid. Besides, this new procedure includes few steps of sample handling, and therefore constitutes an advantage in preventing possible modifications in sample chemical composition. The proposed method is an alternative route for obtaining a fraction that is most representative of those WSOC that are highly conjugated compounds in atmospheric particles. This method has the advantage of isolating and characterising the most hydrophilic compounds, which were not isolated by the method of Varga *et al.* (2001), and that are retained in the XAD-4 resin. Furthermore, with this new methodology higher recoveries of the water-soluble organic matter are obtained, ranging from 63–71%, and only 4–9% of the total WSOC is excluded from the tandem XAD-8/XAD-4 resins. Additionally, the powders obtained after freeze-drying the XAD-8 and XAD-4 eluates constitute another advantage of the present methodology, since they can be kept for a long time for future detailed chemical characterisation.

One interesting feature of the results reported Table III is the different yields obtained through DOC and UV_{250} measurements. In what concerns the XAD-8 fraction, higher percentages of retention and recovery are obtained when assessing organic matter content by UV_{250}. The opposite situation was verified for the XAD-4 fraction, being those percentages higher when organic matter content is assessed

Table IV. Ratio E_{250}/E_{365} of the aqueous extracts, XAD-8 and XAD-4 eluates and XAD-8 and XAD-4 effluents

Fractions	Rural-summer	Rural-autumn	Urban/oceanic
Aqueous Extract	8.7	6.4	6.7
XAD-8 Eluate	8.9	6.1	5.8
XAD-8 Effluent	9.1	13.0	19.8
XAD-4 Eluate	15.1	11.2	13.1
XAD-4 Effluent	6.6	7.8	23.6

through DOC measurements. These features are the end result of the enrichment of the XAD-8 fraction in organic compounds with highly conjugated π bond systems during the first concentration stage of the original WSOC fraction. Therefore, their relative contribution to absorbance at 250 nm is higher than that of DOC, giving higher percentages of organic matter retention and recovery when assessed through UV_{250} measurements. In the case of the XAD-4 fraction, the content of the chromophores responsible for absorbance at 250 nm is lower. Thus, the percentages of organic matter retention and recovery are improved when assessed through DOC measurements.

The ratio of absorbances at 250 nm and 365 nm (E_{250}/E_{365}) for each fraction is shown in Table IV. This ratio has been correlated with both aromaticity and molecular size of aquatic humic solutes (Duarte *et al.*, 2002; Peuravuori and Pihlaja, 1997). It has been stated that when the quotient E_{250}/E_{365} increases both aromaticity and molecular size of aquatic humic substances decrease. The result of a highly degree of conjugation of π bonds is a shift of UV-VIS absorbance towards higher wavelengths, thus decreasing the quotient E_{250}/E_{365} (Williams and Fleming, 1973). In this work we applied the E_{250}/E_{365} ratio as a preliminary parameter to establish differences between the fractions obtained by the new isolation procedure. It can be observed that both XAD-8 eluate and aqueous extract present similar values of E_{250}/E_{365}, suggesting that not only they hold identical mixtures of compounds, but also that the XAD-8 eluate is the most representative fraction of the original aqueous extract. The XAD-8 effluent presents a higher E_{250}/E_{365} ratio than those of the XAD-8 eluate and aqueous extract, which might indicate a lower degree of conjugation of π bonds in the former fraction. The increase of E_{250}/E_{365} ratio for the XAD-8 effluent also suggest that this fraction is represented by compounds that exhibit a lower molecular size than those retained in the XAD-8 resin during the concentration stage. During the concentration of the XAD-8 effluent into de XAD-4 resin, the conjugation of the few unsaturated structures still existing is enhanced, which might explain the decrease of the quotient E_{250}/E_{365} for the XAD-4 eluates of urban/oceanic and rural-autumn aerosol samples. The XAD-4 effluent of the urban/oceanic has the highest E_{250}/E_{365} ratio, suggesting the presence of very polar and highly hydrophilic compounds with small molecular sizes.

4.3. BLANK SAMPLES: ELUTION WITH 0.1 M NaOH versus 40% MeOH

In this work an additional study of the DOC content yielded from blank samples processed by the new isolation procedure and that already established for aquatic humic substances was also performed. In fact, this new isolation procedure of the WSOC from atmospheric aerosols was adapted from the procedure traditionally applied into the isolation of aquatic humic substances (Malcolm, 1991; Esteves *et al.*, 1995; Boerschke *et at.*, 1996; Santos and Duarte, 1998; Peuravuori *et al.*, 1997, 2002). In the traditional tandem XAD-8/XAD-4 resins isolation procedure, 0.1 M NaOH solution is applied for the back elution of organic matter retained in both XAD-8 and XAD-4 resins directly into a cation-exchange resin (in the H^+ from). The use of a cation-exchange resin constitutes another difference between the traditional isolation procedure and the new developed approach presented in this work. In both experiments, three replicates were performed using in each experiment cleaned resins. Table V reports the results of the three replicates expressed as amount of carbon (in μg) recovered from the resins. Despite exhaustive cleaning of the resins before use, both resins showed some bleeding problems with both 0.1 M NaOH and 40% MeOH solutions. In the case of the XAD-8 resin, the 0.1 M NaOH solution caused a higher variability in the amount of recovered carbon than the 40% MeOH solution. The opposite situation was observed for the XAD-4 resin. In spite of this variability, the use of different solutions for the columns elution does not produce different results in terms of amount of recovered carbon. In fact and as suggested in this new proposed method for the isolation and fractionation of aerosol WSOC, the use of a 40% MeOH solution instead of the 0.1 M NaOH has the advantage of eliminating the use of the cation-exchange resin, thus shortening the period of time for sample treatment. Moreover, the use of a NaOH solution has been associated to irreversible hydrolytic reactions of the organic matter (Aiken, 1988), thus producing a fraction that is not truly representative of the original organic material. It was also verified that the amount of carbon bleeding during the elution step with both 40% MeOH and 0.1 M NaOH solutions was less than 1% of the sample DOC content, which can be considered negligible.

Table V. Amount of carbon (in μg C) recovered from the three replicates of the elution of blank samples from the XAD-8 and XAD-4 resins with 0.1 M NaOH and 40% MeOH solutions

XAD Resin	NaOH 0.1 M (μg C)	MeOH 40% (μg C)
XAD-8	62.3	61.3
	98.3	64.5
	59.6	68.7
XAD-4	55.1	99.5
	70.3	64.5
	53.7	43.3

5. Conclusions

Non-ionic macropourous XAD-8 and XAD-4 resins in tandem were applied for the isolation and fractionation of water-soluble organic compounds (WSOC) from atmospheric aerosols. The main objective was to achieve a representative mixture, free from inorganic ions, of the water-soluble organic matter from atmospheric particles. With this new method the XAD-8 eluate, which accounts to 55–60% of total WSOC, was found to be most representative of those WSOC that are highly conjugated compounds in atmospheric particles. Having a representative mixture is of great importance for the study of the environmental significance of this type of organic matter, because too often the properties of aerosol WSOC are simulated by model compounds, whose characteristics are far from the original organic matter contained in the original samples of atmospheric aerosols. The most hydrophilic and of low molecular size compounds were retained in the XAD-4 resin, accounting for 9% of the total WSOC. The MeOH solution was found to be most suitable for preventing hydrolytic reactions of the organic matter during the elution of the retained organic matter, which can occur by using a NaOH solution as in the traditional isolation procedure of aquatic humic substances.

Another objective of this new methodology was the removal of the inorganic species from the WSOC fraction, which was accomplished by desalting processes. However, during these processes the organic compounds that are weakly adsorbed onto the resins polymers were also removed. In the case of the XAD-8 resin, the desalting fraction is probably represented by very polar and hydrophilic compounds, which were to a certain extent also retained with the most hydrophobic compounds.

By applying either DOC or UV_{250} measurements, different patterns of WSOC distribution are obtained, which are a consequence of the organic matter properties. This feature put into evidence the importance of choosing appropriate criteria to evaluate the efficiency of any isolation procedure.

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