Determination of Polyacrylamide in Soil Waters by Size Exclusion Chromatography

Jianhang Lu, Laosheng Wu,* and Jianying Gan

Abstract

Determination of polyacrylamide (PAM) concentration in soil waters is important in improving the efficiency of PAM application and understanding the environmental fate of applied PAM. In this study, concentrations of anionic PAM with high molecular weight in soil waters containing salts and dissolved organic matter (DOM) were determined quantitatively by size exclusion chromatography (SEC) with ultraviolet (UV) absorbance detection. Polyacrylamide was separated from interferential salts and DOM on a polymeric gel column eluted with an aqueous solution of 0.05 M KH_2PO_4 and then detected at a short UV wavelength of 195 nm. Analysis of PAM concentrations in soil sorption supernatants, soil leachates, and water samples from irrigation furrow streams showed that SEC is an effective approach for quantifying low concentrations (0–10 mg L^{-1}) of PAM in waters containing soil DOM and salts. The method has a lower detection limit of 0.02 μg and a linear response range of 0.2 to 80 mg L^{-1}. Precision studies gave coefficients of variation of $<1.96\%$ ($n = 4$) for >10 mg L^{-1} PAM and $<12\%$ ($n = 3$) for 0.2 to 3 mg L^{-1} PAM.

Increasing application of PAM in soil and water (Sojka and Surapaneni, 2001) has generated the need for a straightforward method to measure the concentration of PAM in waters containing various soil constituents (defined as soil waters in this paper), such as runoff water, irrigation tail water, soil extracts, and soil solutions. Due to the characteristics of soil water samples, the analytical methods to meet this purpose should (i) have a lower detection limit of 1 mg L^{-1} or less and (ii) be immune to or be able to tolerate possible interferences from DOM and dissolved salts (Lu and Wu, 2003). Lentz et al. (1996) developed a flocculation-based procedure for estimating PAM concentration in irrigation water and used it successfully to monitor PAM concentration in furrow streams (Lentz et al., 2002). However, since both DOM and salts can significantly affect the flocculation process (Frenkel et al., 1992; Tarchitzky et al., 1999), this method may not be suitable for quantifying PAM in soil extracts and soil solutions that contain much higher DOM and salts than observed in furrow streams. To overcome interference from DOM, Lu and Wu (2001) adopted the N-bromination method (a PAM analytical technique based on spectrophotometry) (Scoggins and Miller, 1975) as the basic PAM measurement technique and performed a second spectrophotometric determination at a 254-nm wavelength to "correct" the interference from DOM. With this modification, the N-bromination method is able to measure PAM concentration in the presence of DOM, provided that the DOM is structurally homogeneous within a series of assay samples. The method was successfully used to quantify PAM concentration in soil supernatants from batch sorption experiments (Lu et al., 2002). However, if the DOM in water samples had been structurally heterogeneous (for instance, if it had several soil sources), this correction approach would have been invalid (Lu and Wu, 2001). A method that can directly quantify PAM in all types of soil water samples is therefore needed.

Physical methods for PAM quantification, such as turbidimetry and viscosity measurement, are usually not sensitive enough for low concentrations (0–10 mg L^{-1}) of PAM encountered in soil waters (Lu and Wu, 2003). Chemical methods, based on measuring the amount of either amide groups or carboxyl groups of PAM, typically have much higher sensitivity. Nevertheless, since both amide and carboxyl functional groups are also commonly found in the molecular structure of DOM, all chemical methods are inevitably subject to DOM interference. This interference is intolerable when DOM concentration greatly exceeds that of PAM. Therefore, separation of PAM from DOM is necessary for accurate measurement of PAM in soil waters.

Size exclusion chromatography (SEC) has the potential to separate PAM from its interferential substances before detection. It has proved effective in measuring PAM concentrations in waters containing organic matter, such as runoff waters in oil production fields (Beazley, 1985; Hunt et al., 1988; Gharfeh and Moradi-Araghi, 1986) and effluents from coal-washing sites (Leung et al., 1987). It is very likely that SEC could also be used for soil waters. However, these established SEC methods cannot be directly applied to soil water samples because of differences in molecular characteristics (charge, structure, and size) and concentration range of the subject PAM and its interferential substances.

The objective of this study was to develop a method for routine measurement of PAM concentrations in soil waters by SEC. Selection of column, mobile phase, and UV detection wavelength will be discussed. Determination of PAM concentration in soil supernatants, soil leachates, and water samples from irrigation furrow streams was performed to demonstrate the applicability of the SEC technique.

Materials and Methods

Preparation of Polyacrylamide Standards

Anionic PAM, with an average molecular weight (MW) of 12 to 15 Mg mol^{-1}, 21% NH_2 group substituted by OH group, was provided by the Celanese Corp. (Louisville, KY). Stock solutions (100 mg L^{-1}) were prepared by dissolving PAM in deionized water and aging (25°C, dark) for one week to obtain a uniform solution before use. Working standards of PAM

Department of Environmental Science, University of California, Riverside, CA 92521. Received 24 Feb. 2003. *Corresponding author (laowu@mail.ucr.edu).

Published in J. Environ. Qual. 32:1922–1926 (2003).
© ASA, CSSA, SSSA
677 S. Segoe Rd., Madison, WI 53711 USA

Abbreviations: DOM, dissolved organic matter; MW, molecular weight; PAM, polyacrylamide; SEC, size exclusion chromatography; UV, ultraviolet.

(0–20 mg L^{-1}) were prepared by proper dilution of the stock solution with deionized water.

Size Exclusion Chromatography

Analysis of PAM was performed on an Agilent (Palo Alto, CA) 1100 high performance liquid chromatograph (HPLC) system equipped with an autoinjector and a ultraviolet (UV) multiple wavelength detector. A TSK-Gel GMPWXL column (7.8-mm i.d. × 30-cm length; 13-μm particle size; mixed-bed with pore size ranging from 100 to 1000 Å; semirigid porous methacrylate-based polymer gel as main constituent component) purchased from the Tosoh Biosep Corp. (Montgomeryville, PA) was used. The column was eluted at a flow rate of 1.0 mL min^{-1} and an ambient temperature of 25 ± 2°C with a solution of 0.05 *M* KH_2PO_4 (passed through a 0.2-μm filter) as the mobile phase. The wavelength of the UV detector was set at 195 nm. The injection volume was 100 μL. Under these conditions, the retention time for PAM was approximately 5.7 min, and sample output rate was about three per hour. Polyacrylamide concentration was calibrated and quantified based on its peak area.

Sample Pretreatment

Soil water sample containing PAM was first centrifuged for 6 min at 5000 × *g* to remove any possible suspended particles, and was then mixed with a solution of 2.0 *M* KH_2PO_4 at a ratio of 3:1 by volume (water sample to KH_2PO_4 solution) to obtain the same ionic strength with the mobile phase. After that, a 1-mL aliquot was transferred to an autosampler vial for SEC analysis.

Preparation of Soil Supernatant Samples

Soil supernatant samples containing PAM were prepared by the batch equilibrium method used for sorption experiments. Soil samples that encompassed a wide range of texture and organic matter contents were collected from four sites in western United States. They are an Arlington loamy sand (coarse-loamy, mixed, active, thermic Haplic Durixeralf), a Linne clay loam (fine-loamy, mixed, superactive, thermic Calcic Pachic Haploxeroll), a Palouse silt loam (fine-silty, mixed, superactive, mesic Pachic Ultic Haploxeroll), and an Imperial silty clay (fine, smectitic, calcareous, hyperthermic Vertic Torrifluvent). These soil samples were collected from the top-10-cm layer of the profiles, air-dried, and ground to pass through a 1-mm sieve. Their textural and chemical properties can be found elsewhere (Lu and Wu, 2001). In the sorption experiment, 1.5 g of soil was added to 30 mL of PAM solution (20 mg L^{-1} prepared with deionized water) in a 50-mL glass bottle and shaken for 36 h. The mixture was centrifuged for 10 min at 3000 × *g*, and a subsample of the supernatant was withdrawn for analysis of PAM concentration by SEC and by the modified N-bromination method (Lu and Wu, 2001), respectively. In the N-bromination method, individual DOM correction curves for each soil were established.

Preparation of Soil Leachate Samples

Two soils, the Arlington loamy sand and the Linne clay loam, were used. Each soil was packed into a short plastic column (5.1-cm i.d. × 3-cm height). The soil in the Arlington loamy sand column had a bulk density of 1.43 g cm^{-3} and a pore volume of 25.3 cm^3, while the soil in the Linne clay loam column had a bulk density of 1.10 g cm^{-3} and a pore volume of 32.5 cm^3. The soil columns were leached with a PAM solution (10 mg L^{-1}) with a constant head of 3 cm. The PAM solution was prepared in tap water (Riverside, CA) with an electrical conductivity of 0.67 dS m^{-1} and a sodium adsorption ratio of 1.12. The leachates were collected at a predetermined volume interval. Each fraction of the leachate was mixed thoroughly and a subsample was removed for PAM quantification by SEC.

Collection of Water Samples from Irrigation Furrow Streams

The samples were collected from a field located in the field station of University of California Desert Research & Extension Center (Holtville, CA). The surface soil was a silt clay loam and was dry before irrigation. The furrows were 380 m long with 0.15% slope. Polyacrylamide was dissolved and mixed well into the irrigation water before reaching the furrows and had an initial concentration of 3 mg L^{-1} in one application and 1 mg L^{-1} in another. For each furrow, five sampling points located at 6.1, 91.2, 182.4, 273.6, and 364.8 m downstream from the inflow end were chosen. To evaluate how the PAM concentration changes in the advancing furrow stream, water samples were taken from each sampling point when the flow first passed through that point. The water samples were centrifuged within 30 min after field sampling to remove sediments and stored in a refrigerator before analysis.

Results and Discussion

Optimization of Size Exclusion Chromatography Conditions

The major difficulty in quantification of PAM by SEC lies in the isolation of PAM from its interferential substances. The SEC method separates different substances on the basis of molecular hydrodynamic volume (or size). Based on the results of light-scattering measurements on PAMs with similar MW and charge density (Muller et al., 1979; Hecker et al., 1999), the gyration radius of the anionic PAMs commonly used in soil water conservation is roughly in the range of 90 to 300 nm, depending on the ambient ionic strength. Separation of PAM from salts and small organic molecules, such as fulvic acids, can be readily achieved due to the vast differences in their molecular sizes. However, separation of PAM from humic substances, which are also high MW polymers (MW ranging from several hundred to several hundred thousand daltons) (Beckett et al., 1987; Stevenson, 1994), can only be achieved under certain experimental conditions. Selections of column and of mobile phase are essential to the success of their separation.

Selection of Column. The chemistry and pore size of column packing are two important factors in selection of a column. Two kinds of packing have been used for quantification of aqueous PAMs, that is, surface glycerol-modified silica (Beazley, 1985; Gharfeh and Moradi-Araghi, 1986) and methacrylate-based hydrophilic polymer gel (Leung et al., 1987; Papazian, 1990). Surface-modified silica columns have better mechanical strength but usually have lower resolution power and are much more susceptible to non-size-exclusion interactions (ion exchange, ion exclusion, and sorption) that cause peak tailing (Meehan, 1995). At present, the largest pore size in the commercially available aqueous SEC columns is about 1000 Å. Since the molecular size of the PAM commonly used in soil water conservation is about 900 to 3000 Å, PAM is likely to be excluded

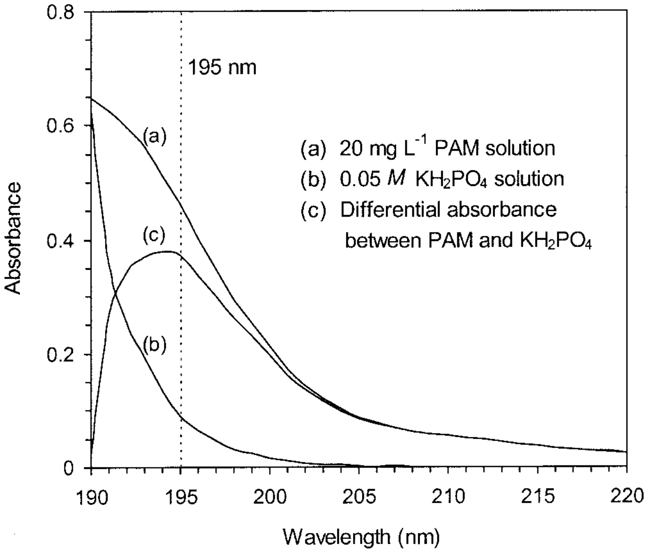

Fig. 1. Ultraviolet absorbance spectra of 20 mg L^{-1} polyacrylamide (PAM) and 0.05 *M* KH_2PO_4 solutions.

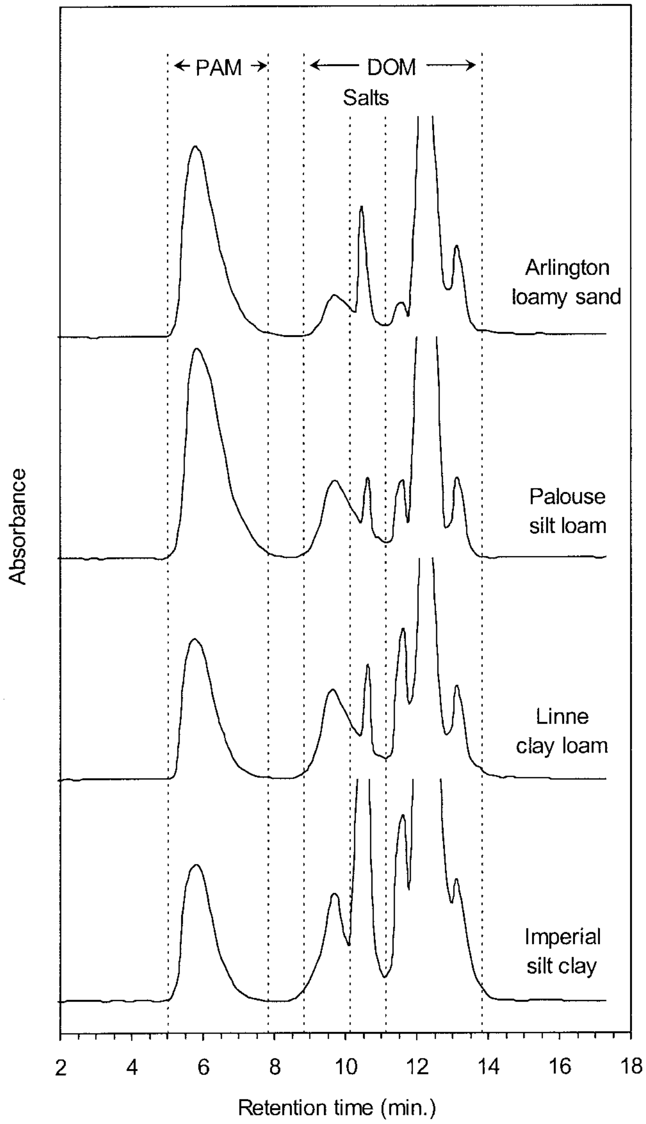

Fig. 2. Size exclusion chromatograms of polyacrylamide (PAM) in four soil supernatants. Chromatographic conditions: 7.8-mm-i.d. × 30-cm-long TSK-Gel GMPWXL column; 0.05 *M* KH_2PO_4 mobile phase; 1.0 mL min^{-1} flow rate; 100-μL injection volume; and ultraviolet (UV) detection at 195 nm.

from all the pores of the column packing. Therefore, a column with a pore size larger than the molecular size of DOM should be chosen, so that all DOM, including humic substances (the portion of DOM with the largest molecular size), can readily diffuse into the pores and thus be eluted at a later time than PAM. Small-angle X-ray scattering measurements showed that the molecular size of aquatic humic substances was several to several tens of Angstroms (Thurman et al., 1982). However, since both the modified silica packing and the polymer gel packing contain some residual ionic groups in their surfaces, humic substances may be excluded from some of the pores and be eluted earlier than predicted based on their molecular size due to ion-exclusion interaction. Such phenomena have been observed for phenolcarboxylic acids, which have a simpler but similar structure to humic acids (De Nobili and Chen, 1999). The SEC columns used for investigating MW distribution of humic substances usually have a pore size of about 100 to 250 Å (De Nobili and Chen, 1999; Pfeifer et al., 2001; Piccolo et al., 2001). In the case of PAM quantification, humic substances are expected to be eluted later. Therefore, a column with larger pore size (250 Å or up) is expected to ensure a good effusion of humic substances. In this study, a methacrylate-based polymer gel column with a pore size up to 1000 Å (mixed-bed) was used.

Selection of Mobile Phase. The hydrodynamic sizes of PAM and some DOM molecules, both carrying negative charges, largely depend on the ambient ionic strength. Adjustment of ionic strength in the eluting solution with appropriate salt is necessary to control their hydrodynamic volume and to minimize nonexclusion effects. Salts previously used for these purposes include Na_2SO_4, $NaClO_4$, and KH_2PO_4. In our preliminary experiment, KH_2PO_4 solution was found most suitable. Na_2SO_4 has a high UV absorbance in wavelengths shorter than 200 nm. $NaClO_4$ has low absorbance, but the shape and retention time of the PAM peak is not as stable as with KH_2PO_4. The appropriate concentration of KH_2PO_4 in the mobile phase is 0.05 *M*; lower concentrations cause an inefficient separation between PAM and DOM in some soil water samples and higher concentrations are not advisable due to increasing background absorbance.

KH_2PO_4 solution was also added to the sample before SEC analysis to minimize the ionic strength difference between the sample and the mobile phase. This was found to stabilize the retention time of PAM peak and to improve its shape. Moreover, the negative-polarity water peak in the chromatogram was avoided.

Selection of Ultraviolet Detection Wavelength. Both refractive index difference and UV absorbance have been used for PAM detection in the past (Onda et al., 1980; Beazley, 1985). An ultraviolet detector operating at short wavelengths (190–215 nm) was found to be more sensitive than a refractive index difference detector in trace PAM analysis (Hunt et al., 1988). Since the UV absorbance of PAM increases considerably as wavelength decreases in the range of 190–215 nm (Fig. 1), shorter detection wavelengths give higher detection sensitivity. However, the background absorbance caused by the KH_2PO_4 mobile phase also increases as wavelength decreases. To ensure a high detection sensitivity and a good baseline stability, we set our UV detector to 195 nm where the largest differential absorbance between PAM and KH_2PO_4 solution was found (Fig. 1).

Polyacrylamide Analysis in Soil Supernatants

Typical chromatograms of PAM in sorption supernatants of the four soils are presented in Fig. 2. Polyacrylamide was eluted in about 5.7 min as a single, slightly skewed and tailed peak, while interferential substances, DOM and salts, were eluted at a later time of about 9 to 14 min. Due to different characteristics of soil constituents, each water sample had its own unique DOM and salt peaks (Fig. 2).

Since PAM is readily separated from DOM and salts, its concentration can be accurately determined based on the peak area. The skewed shape of the PAM peak reflects the MW distribution of the PAM, and the tailing is caused by a small amount of low MW components in the PAM formulation. Peaks of PAM from different manufacturers varied slightly in skewness and tailing (data are not shown). Elimination of the tailing can improve the precision of PAM quantification. While using a column with smaller pore size (for instance, 300–500 Å) than the one used in this study may exclude the low MW portion of PAM more efficiently and thus reduce the skewing and tailing of the peak, one could risk insufficient resolution between PAM and DOM.

Analysis of PAM standard solutions of various concentrations showed that, under the chromatographic conditions used in this study, the linear response range of PAM was 0.2 to 80 mg L^{-1}. The lower detection limit, using a signal-to-noise ratio of 5, is about 0.02 μg, corresponding to a PAM concentration of 0.2 mg L^{-1} with an injection volume of 100 μL.

The measured PAM concentrations in the sorption supernatants of the four soils are listed in Table 1. For each sample, four replicates performed on separate days were done to show day-to-day variations. Polyacrylamide concentrations in the sorption supernatants were different in these soils, ranging from 10.19 to 17.59 mg L^{-1}, with coefficients of variation less than 1.96% (n = 4). These concentration values agreed well with the results of the N-bromination method (Table 1). Comparison of the coefficients of variation showed SEC has better precision than the N-bromination method, partially because less volumetric operations were involved in the SEC analysis procedure.

Polyacrylamide Analysis in Soil Leachates

When the same soil and comparable solution-to-soil ratios were used, the above-mentioned soil supernatant samples had similar compositions of DOM and salts. However, water samples leached from the same soil in a time sequence may have different compositions of DOM and salts. Compared with later leachates, the DOM in earlier leachates contains more easily dissolved components that are lower in MW and have greater numbers of hydrophilic groups. In other words, DOM in leachates collected at different times from the same soil may be structurally heterogeneous. This makes analysis of PAM concentration in soil leachates by the N-bromination methods (Lu and Wu, 2001) unsuitable. However, the heterogeneity of DOM in soil water samples should not affect quantification by SEC since PAM is detected after it is separated from DOM.

Table 1. Concentration of polyacrylamide (PAM) in soil supernatants determined by size exclusion chromatography and by N-bromination method.

	Size exclusion chromatography		N-bromination method	
Soils	**Mean ± SD†**	**CV**	**Mean ± SD**	**CV**
	mg L^{-1}	**%**	**mg L^{-1}**	**%**
Arlington loamy sand	**16.44 ± 0.27**	**1.66**	**16.31 ± 0.40**	**2.46**
Linne clay loam	**10.60 ± 0.12**	**1.24**	**9.98 ± 0.28**	**2.81**
Imperial silty clay	**10.19 ± 0.20**	**1.96**	**10.51 ± 0.19**	**1.76**
Palouse silt loam	**17.59 ± 0.14**	**0.79**	**17.19 ± 0.45**	**2.63**

† Mean values are averages of four measurements.

Plots of PAM concentration versus leachate volume through the Arlington sandy loam and the Linne loam are given in Fig. 3. As expected, PAM has very low permeability in both soils. For the 3-cm-tall soil column, even after 50 pore volumes, the PAM concentration in the leachates was still less than half of the applied concentration (10 mg L^{-1}). The precision of determination of PAM concentrations in soil leachates is satisfactory, as shown by the small error bars in Fig. 3., indicating that the SEC technique is appropriate for quantifying low concentrations (0–10 mg L^{-1}) of PAM commonly found in soil waters.

Polyacrylamide Analysis in Waters from Furrow Streams

The method was further tested to determine the PAM concentration in waters from irrigation furrow streams.

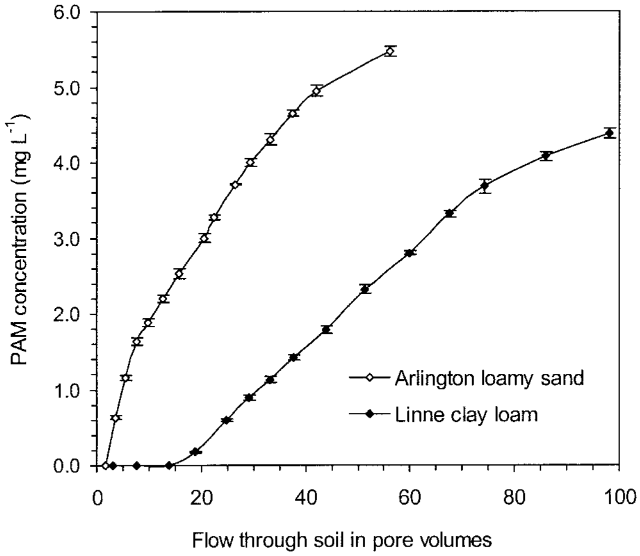

Fig. 3. Concentration of polyacrylamide (PAM) in soil leachate as a function of flow volume through soil columns. Each point is the mean of three individual measurements. Error bars represent the standard deviations of the means.

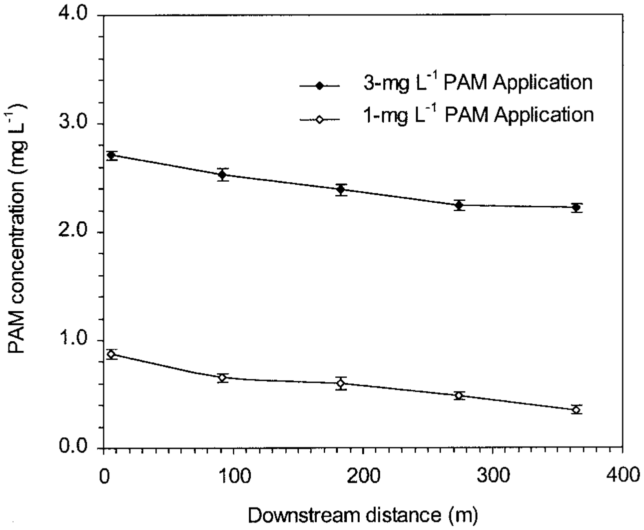

Fig. 4. Polyacrylamide concentrations in furrow streams during water advancing. Each point is the mean of three individual measurements. Error bars represent the standard deviations of the means.

The concentrations of PAM in furrow streams were plotted versus flow distance in Fig. 4. Polyacrylamide concentration in furrow streams decreases slowly as the water flow advances in the furrows. The precision of the analysis, as shown by the error bar in Fig. 4, becomes slightly lower when PAM concentration is very low (0–3 mg L^{-1}), but it is still good enough to show the trend of PAM concentration change. The coefficients of variation for these analyses were <12% (n = 3). This demonstrates the utility of the SEC procedure for monitoring PAM concentration in field water samples.

Conclusions

Soil DOM inevitably causes interferences in all available physically and chemically based methods for measuring PAM concentration in soil waters. In this study a SEC with UV detection technique was developed for quantifying PAM (0–10 mg L^{-1}) in soil waters. Separation of PAM from interferential DOM and salts was achieved on a TSK-GEL polymer column with appropriate adjustments of ionic strength in the mobile phase. Aqueous SEC columns used for this purpose should have an appropriate pore size in their packing, which will exclude the majority of the PAM molecules while keeping the column totally permeable to DOM and salts. The use of a UV detector operating at short wavelength of 195 nm, where the maximum differential absorbance between PAM and the mobile phase (0.05 M KH_2PO_4) was found, ensured high sensitivity for PAM detection. Analysis of PAM concentration in soil supernatants, soil leachates, and water samples from irrigation furrow streams showed that the technique had satisfactory sensitivity, precision, and reproducibility. The methodology described here offers considerable promise for use in research work on the fast-growing field of PAM technology in soil and water conservation.

References

Beazley, P.M. 1985. Quantitative determination of partially hydrolyzed polyacrylamide polymers in oil field production water. Anal. Chem. 57:2098–2101.

Beckett, R., Z. Jue, and J.C. Giddings. 1987. Determination of molecular weight distribution of fulvic and humic acids using flow field-flow fractionation. Environ. Sci. Technol. 21:289–295.

De Nobili, M., and Y. Chen. 1999. Size exclusion chromatography of humic substances: Limits, perspectives and prospectives. Soil Sci. 164:825–833.

Frenkel, H., M.C. Fey, and G.J. Levy. 1992. Organic and inorganic anion effects on reference and soil clay critical flocculation concentration. Soil Sci. Soc. Am. J. 56:1762–1766.

Gharfeh, S.G., and A. Moradi-Araghi. 1986. Determination of anionic high-molecular-weight water-soluble polymers by size-exclusion chromatography. J. Chromatogr. 366:343–350.

Hecker, R., P.D. Fawell, A. Jefferson, and J.B. Farrow. 1999. Flow field-flow fractionation of high-molecular-mass polyacrylamide. J. Chromatogr. A 837:139–151.

Hunt, J.A., T.S. Young, D.W. Green, and G.P. Willhite. 1988. Size-exclusion chromatography in the measurement of concentration and molecular weight of some EOR polymers. SPE Reservoir Eng. 3:835–841.

Lentz, R., R.E. Robert, and B.E. Mackey. 2002. Fate and efficacy of polyacrylamide applied in furrow irrigation: Full-advance and continuous treatments. J. Environ. Qual. 31:661–670.

Lentz, R.D., R.E. Sojka, and J.A. Foerster. 1996. Estimating polyacrylamide concentration in irrigation water. J. Environ. Qual. 25:1015–1024.

Leung, R.W.M., R.N. Pandey, and B.S. Das. 1987. Determination of polyacrylamides in coal washery effluents by ultrafiltration/size-exclusion chromatography-ultraviolet detection techniques. Environ. Sci. Technol. 21:476–481.

Lu, J.H., and L. Wu. 2001. Spectrophotometric determination of polyacrylamide in waters containing dissolved organic matter. J. Agric. Food Chem. 49:4177–4182.

Lu, J., and L. Wu. 2003. Polyacrylamide quantification methods in soil conservation study. J. Soil Water Conserv. (in press).

Lu, J.H., L. Wu, and J. Letey. 2002. Effects of soil properties and water quality on sorption of anionic polyacrylamide by soils. Soil Sci. Soc. Am. J. 66:578–584.

Meehan, E. 1995. Semirigid polymer gels for size exclusion chromatography. p. 25–46. *In* C.S. Wu (ed.) Handbook of size exclusion chromatography. Marcel Dekker, New York.

Muller, G., J.P. Laine, and J.C. Fenyo. 1979. High-molecular-weight hydrolyzed polyacrylamides. I. Characterization. Effect of salts on the conformational properties. J. Polym. Sci. Polym. Chem. Ed. 17:659–672.

Onda, N., K. Furusawa, N. Yamaguchi, M. Tokiwa, and Y. Hirai. 1980. Gel permeation chromatography using controlled-porosity glass. II. Polyacrylamide aqueous solutions. J. Appl. Polym. Sci. 25:2363–2372.

Papazian, L.A. 1990. A pH effect in the HPSEC separation of polyacrylamide-based copolymers. J. Liq. Chromatogr. 13:3389–3398.

Pfeifer, T., U. Klaus, R. Hoffmann, and M. Spiteller. 2001. Characterization of humic substances using atmospheric pressure ionization and electrospray ionization mass spectrometry combined with size-exclusion chromatography. J. Chromatogr. A 926:151–159.

Piccolo, A., P. Conte, and A. Cozzolino. 2001. Chromatographic and spectrophotometric properties of dissolved humic substances compared with macromolecular polymers. Soil Sci. 166:174–185.

Scoggins, M.W., and J.W. Miller. 1975. Spectrophotometric determination of water soluble organic amides. Anal. Chem. 47:152–154.

Sojka, R.E., and A. Surapaneni. 2001. Potential use of polyacrylamide (PAM) in Australian agriculture to improve off- and on-site environmental impacts and infiltration management [Online]. Available at http://kimberly.ars.usda.gov/Sojka/Oz/OzPAMUSA.htm (verified 22 Apr. 2003). USDA, Kimberly, ID.

Stevenson, F.J. 1994. Humus chemistry, genesis, composition, reactions. 2nd ed. John Wiley & Sons, New York.

Tarchitzky, J., Y. Golobati, R. Keren, and Y. Chen. 1999. Wastewater effects on montmorillonite suspensions and hydraulic properties of sandy soils. Soil Sci. Soc. Am. J. 63:554–560.

Thurman, E.M., R.L. Wershaw, R.L. Malcolm, and D.J. Pinckney. 1982. Molecular size of aquatic humic substances. Org. Geochem. 4:27–35.