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Determination of phosphonic acid breakdown products by high-performance liquid chromatography after derivatization

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Abstract

A new HPLC method for the determination of oxidative breakdown products of aminopolyphosphonates is presented. The phosphonate nitrilotrismethylenephosphonic (NTMP) acid undergoes catalytic oxidation by molecular oxygen in the presence of manganese(II). The two diphosphonates iminodimethylenephosphonic acid (IDMP) and formyliminodimethylenephosphonic acid (FIDMP) are formed. The analytical method employs the derivatization of the aldehyde group in FIDMP by 2,4-dinitrophenylhydrazine and of the imine group in IDMP by 9-fluorenyl methylchloroformate. The two derivatives are quantified in separate runs using the same acidic phosphate–acetonitrile eluent with detection at 370 nm for FIDMP and 260 nm for IDMP. The detection limit for FIDMP is 0.01 μM, for IDMP 0.02 μM. The method is suitable for the determination of the breakdown products in wastewater.

Keywords: Derivatisation, LC; Water analysis; Phosphonic acid; Organophosphorous compounds

1. Introduction

Phosphonate chelating agents are used in a wide range of applications, including scale and corrosion inhibition, metal finishing, ore recovery, oil drilling, pulp, paper and textile production, cleansing and laundry operations and agriculture [1]. Phosphonates are not biodegraded during conventional wastewater treatment [2,3] but exhibit nevertheless a significant removal [4]. This is caused by their very strong adsorption onto biosolids [5] and the iron oxides [6,7] formed in tertiary treatment.

It has recently been shown that aminopolyphosphonates are rapidly degraded in the presence of Mn(II) and molecular oxygen [8]. This metal-catalyzed degradation of phosphonates might be an important process governing the fate of phosphonates in process, waste and natural waters. The Mn(II)-catalyzed degradation of nitrilotrismethylenephosphonic acid (NTMP) yields two diphosphonic acid breakdown products, IDMP (iminodimethylenephosphonic acid) and FIDMP (formyl iminodimethylene phosphonic acid).

The standard method for determining phosphonates is ion-chromatography followed by postcolumn reaction with Fe(III) and detection of the Fe(III) complexes at 300–330 nm [9,10]. This method has a detection limit of about 2–10 μM. Other methods have been developed based on postcolumn oxidation of the phosphonate to phosphate and detection of

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phosphate with the molybdenum blue method [11]. Derivatization of the phosphonic acid groups with diazomethane and separation and detection by HPLC–MS has also been described [12]. All these methods are, however, not applicable to natural waters either due to their high detection limits or due to interferences by major cations and anions in the water. An ion-pair HPLC method with precolumn formation of the Fe(III) complexes has been described by which phosphonates can be measured with a detection limit of 0.05 μ*M* in natural waters and wastewaters [13]. The fate of phosphonates during wastewater treatment was studied using this method [4]. The method, however, is not able to quantify diphosphonic acids such as the above mentioned breakdown products of NTMP.

The aim of this work was to develop analytical methods for the determination of IDMP and FIDMP in natural waters based on derivatization of the aldehyde group in FIDMP by 2,4-dinitrophenylhydrazine (DNPH) and of the imine group in IDMP by 9-fluorenyl methylchloroformate (FMOC) (Fig. 1). DNPH is widely used for the derivatization of carbonyl compounds [14,15] and FMOC is a standard reagent for the analysis of amino and imino acids [16,17] and has also been used for aminophosphonic acids [18].

2. Experimental

2.1. Reagents and chemicals

Water was obtained from a Milli-Q system (Millipore). All chemicals were obtained from Fluka (Switzerland) if not otherwise specified. Pure FIDMP is not available; it was prepared by the oxidation of NTMP by molecular oxygen in the presence of Mn(II) [8]. NTMP (10 μ*M*) and $Mn(II)Cl_2$ (10 μ*M*) were reacted at pH 6 for 20 min in air-saturated water. The mixture was then passed through a Dionex On-Guard H cartridge to remove Mn(II). Residual NTMP was measured by HPLC [13], IDMP was quantified using an available standard (Fluka). The FIDMP concentration was then calculated by subtracting the measured concentration of NTMP and IDMP from the initial NTMP concentration.

A 1 *M* borate buffer was prepared from boric acid adjusted with sodium hydroxide to pH 6.2. This solution diluted five times in water gives pH 7.7. The FMOC reagent was prepared by dissolving 155 mg of fluorenylmethyl chloroformate (Fluka) in 40 ml acetone to give a concentration of 15 m*M*. The DNPH reagent was prepared by dissolving 20 mg 2,4-dinitrophenylhydrazine (Fluka) in 15 ml of a

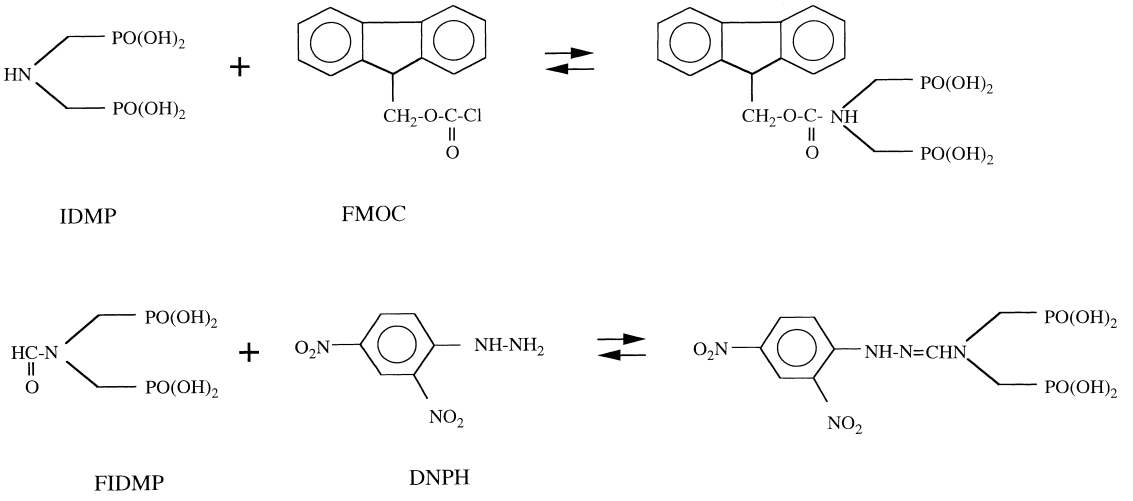

Fig. 1. Scheme of the reaction of IDMP with FMOC and of FIDMP with DNPH.

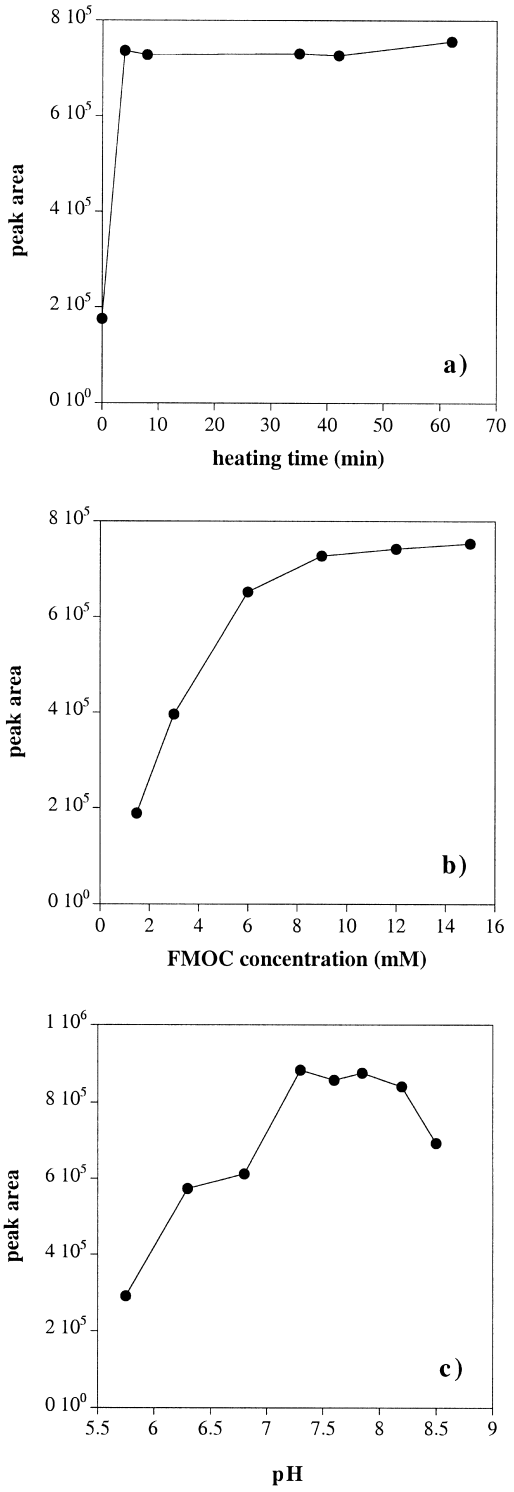

Fig. 2. Influence of heating time at 60°C (a), FMOC concentration (b) and pH (c) on the derivatization of 10 μ*M* IDMP with FMOC.

solution of concentrated HCl–water–acetonitrile (2:5:1, v/v/v).

2.2. HPLC

A Jasco high-performance liquid chromatographic system (PU-980) equipped with a UV spectrophotometric detector (UV 970) set at 370 nm (FIDMP) or 260 nm (IDMP) and a 851-AS autosampler was used. A sample loop of 200 μl was used. The HPLC separations were performed on a LiChrosphere 100 (RP-18,125×4 mm, 5 μm) column with a mobile phase consisting of 0.1 *M* NaH_2PO_4 and 0.06 *M* H_3PO_4 with a pH of 2.6. The acetonitrile concentration for IDMP was 21%. For FIDMP the following acetonitrile gradient was used: 10–30% in 12 min, 2 min at 30%, 30–10% in 1 min, 7 min at 10%. The flow-rate was 1 ml/min at room temperature.

2.3. Derivatization of IDMP

To 0.8 ml of water sample (e.g. wastewater) were added 0.2 ml borate buffer and 1.0 ml of the FMOC reagent. The sample was heated 10 min on a heating block at 60°C. After cooling to room temperature, 2 ml of dichloromethane was added, the sample was shaken, centrifuged and the aqueous layer transferred to a vial and 200 μl was injected into the HPLC. Samples containing calcium at concentrations above 1 m*M* formed a white precipitate after FMOC addition. A 1-ml volume of fresh sample was passed through a cation-exchange resin (Dionex On-Guard H cartridge) before derivatization to remove Ca and Mg and 0.8 ml was derivatized as described above.

2.4. Derivatization of FIDMP

A separate sample of water (1 ml) was derivatized by adding 20 μl of DNPH reagent solution. The sample (200 μl) can be injected immediately into the HPLC.

3. Results and discussion

3.1. Derivatization of IDMP

The derivatization of amino and imino acids by

FMOC is complete within 30 s at room temperature [17]. The derivatization of IDMP, however, is much slower. A reaction time of <1 min at room temperature is not sufficient for a complete derivatization of IDMP. After heating for 10 min at 60°C a maximal conversion to the derivative is achieved (Fig. 2a). The FMOC concentration of 15 m*M* used by Einarsson et al. [17] for amino acids was found to be optimal for IDMP, too (Fig. 2 b). The effect of pH on the derivatization is shown in Fig. 2c. The pH of 7.7 reported for amino acid derivatization [17] was also found to be optimal for IDMP derivatization. Unlike Einarsson et al. [17] who used pentane to extract unreacted FMOC and acetone from the reaction mixture, dichloromethane was used here. Due to the high density of dichloromethane, the aqueous layer can easily be removed from the organic phase. The additional cation-exchange treatment of natural samples removes any interference from cations such as Ca and Mg and is therefore used with all natural samples.

3.2. Derivatization of FIDMP

The derivatization of FIDMP with DNPH is very rapid and not sensitive to acid or base concentrations in the sample of 0.05 *M*. A final pH of the reaction mixture between 1 and 2.5 after addition of DNPH reagent yields the same peak area. The amount of DNPH added is also not very crucial as long as it is in excess of FIDMP. Cations like Ca^{2+} at concentrations of 0.1 *M* or less do not interfere with the reaction. No pretreatment for natural samples is therefore needed.

3.3. HPLC

Fig. 3 shows chromatograms for IDMP and FIDMP in Milli-Q water. The analytes are well separated from the reagent peaks. For FIDMP a gradient has been used to flush out the reagent peak of DNPH. Because the reagent peak of FMOC elutes before the analyte, an isocratic elution can be used for IDMP determination. The chromatograms of FIDMP show two peaks for FIDMP with the same ratio of the peak areas for all concentrations. For quantification the first large peak was always used.

A plot of the peak area versus the concentration of FIDMP is linear from 0.2 to 2.5 μ*M* with a correlation coefficient r^2 of 0.999 and from 0.02 to 0.2 μ*M* with a correlation coefficient r^2 of 0.999. A plot of the peak area versus the concentration of IDMP is linear from 0.2 to 3 μ*M* with a correlation coefficient r^2 of 0.98 and from 0.5 to 10 μ*M* with a correlation coefficient r^2 of 0.997. The detection limit for FIDMP is 0.01 μ*M* and for IDMP 0.02 μ*M* ($S/N=3$).

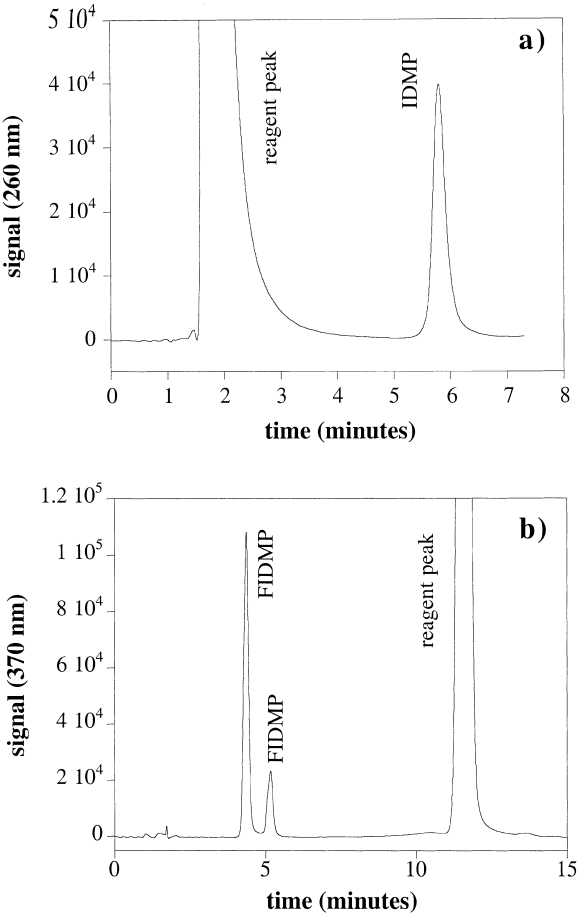

Fig. 3. Chromatograms of 10 μ*M* IDMP (a) and 2 μ*M* FIDMP (b).

3.4. Analyses

The methods have been applied to the analysis of FIDMP and IDMP in wastewater treatment plants (WWTP). Samples were measured from WWTP that receive wastewater from textile industry, which uses large amounts of phosphonates. The samples for FIDMP analysis were simply filtered through 0.45-

μm and derivatized with DNPH. Fig. 4a shows that more FIDMP was detected in the influent than in the effluent. Standard addition of FIDMP confirmed its presence. A sample of drinking water gave no detectable FIDMP peak (lowest chromatogram). The concentration of FIDMP in the influent was 0.08 μ*M* and in the effluent 0.01 μ*M*. The removal efficiency for this compound is therefore comparable to the removal efficiency of the phosphonate DTPMP of over 80% observed in the same WWTP [4]. IDMP was also detected at a concentration of 0.49 μ*M* in the influent sample (Fig. 4b) but not in the effluent. Analysis of a second WWTP that receives wastewater from textile industry showed a concentration of IDMP in the influent of 0.3 μ*M* and no detectable concentration in the effluent. The concentration of FIDMP in this WWTP was 0.015 μ*M* in the influent and below the detection limit in the effluent.

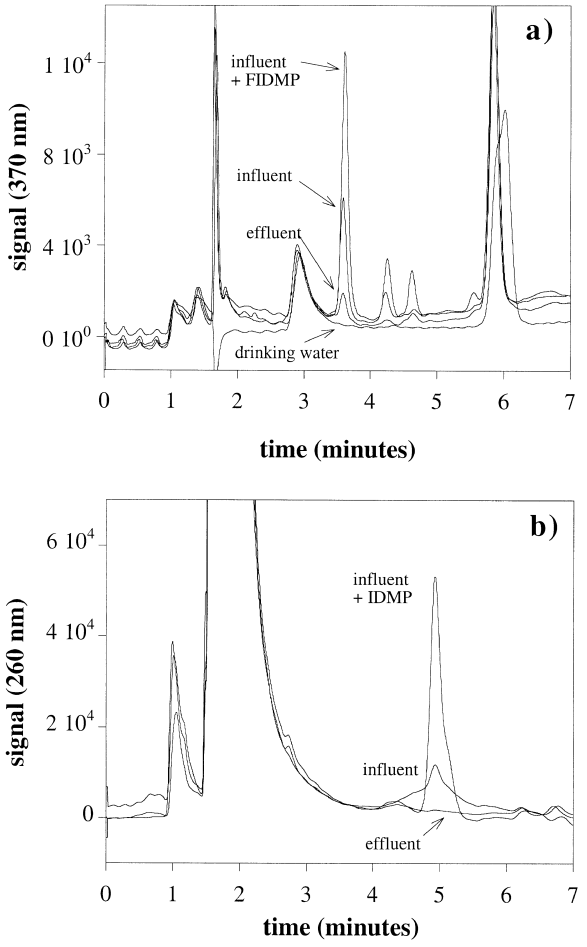

Fig. 4. Analysis of FIDMP (a) and IDMP (b) in samples from the influent and effluent of a wastewater treatment plant receiving wastewater from textile industry. Measured condentrations: FIDMP influent 0.08 μ*M*, effluent 0.01 μ*M*; IDMP influent 0.49 μ*M*.

Laboratory experiments have shown that oxidation of NTMP to FIDMP and IDMP in the presence of Mn(II) and molecular oxygen is very rapid at pH around 6 [8]. The results from WWTP presented here prove that degradation of NTMP to the two products FIDMP and IDMP took place during application in textile industry or in wastewater. The removal of these two breakdown products during water treatment is, however, comparable to that of the parent phosphonate NTMP.

4. Conclusions

Two oxidative breakdown products of NTMP can be derivatized using DNPH for FIDMP and FMOC for IDMP to give derivatives suitable for separation by reversed-phase HPLC. The method is applicable to the determination of the compounds in wastewater at trace levels.

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