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Liquid chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry determination of *N*-methylpyrrolidinone in riverine biofilms

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Abstract

A rugged procedure utilizing reversed-phase liquid chromatography with positive-ion electrospray ionization mass spectrometry (LC–MS) along with tandem MS is described for the quantification and confirmation of *N*-methylpyrrolidinone (NMP) in methanolic extracts of riverine biofilm. The LC–MS method provided a 100-fold improvement in detection limits (2 ng g^{-1} with a repeatability of 80–95% based on triplicate analyses) compared to a conventional LC–UV detection procedure and was applicable to quantitative analysis of biofilm samples with little or no clean up. Under low-energy collision induced dissociation (CID) conditions (17 V, laboratory frame of reference, with argon as the collision gas), two product-ions of the $[M+H]^+$ ion were formed at m/z 69 $[MH\text{-}CH_3NH_2]^+$ and m/z 58 $[MH\text{-}CH_3NCH]^+$ with relative abundances of 30% and 5%, respectively. These CID transitions were used to demonstrate that biofilm uptake of a photocatalytically-generated mixture of NMP was rapid once acclimation was achieved.

Keywords: Riverine biofilms; *N*-Methylpyrrolidinone

1. Introduction

Biofilms are natural microbiological assemblies and are ubiquitous in aquatic environments. The binding of these assemblies to surfaces in the aquatic environment is due to extracellular polymeric substances (EPSs), which are largely polysaccharides in composition [1,2]. The EPSs serve other functions, including protection against environmental fluctuations, anti-microbial agents, and predation, as well as the localization of extracellular enzymes [3–8]. They can sorb nutrients, metals, and organic contaminants in the aqueous phase [3,6,7,9–15]. Natural biofilms can also degrade certain organic contaminants [10,11], and biofilms have been studied in the context of reducing overall dissolved organic carbon content in surface water supplies [16]. These findings suggest that biofilm uptake could also be a valuable component in the treatment of small-scale contamination such as pesticide rinsates. However, the threshold concentration for sloughing and destruction of the biofilm can be quite low in the case of many

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organic contaminants [9]. Pretreatment of a contaminated water by, for example, TiO_2 photo-oxidation [17,18], to reduce the toxicity of the influent by lowering levels of the original contaminant and/or converting it to a less toxic form would be an important step in a biofilm treatment scheme.

Critical to the development of this technology is the determination of contaminants and intermediates present in the biofilm and effluent. Previous work in our group has centered around development of a tandem mass spectrometry (MS) method employing direct probe introduction of biofilms with no prior chromatographic separation, for the determination of contaminants in biofilms and biota [10,19,20]. While this technique worked well for the identification and confirmation of selected transformation products in biofilms, it was noted that the technique could likely be refined using chromatographic separation of sample extracts, prior to MS detection. The authors also alluded to alternative approaches using high-resolution MS without tandem MS.

The present work describes an approach utilizing a liquid chromatography (LC)–MS and LC–MS–MS procedure based on reversed-phase LC with electrospray ionization (ESI) of methanolic extracts of riverine biofilm samples. The liquid chromatographic separation utilized in the current procedure can be regarded as a refinement of the previous MS–MS method in which a direct insertion probe was used [10,20]. While the earlier method facilitated the introduction of small sample sizes so that there was no need for extensive labor-intensive collection in the field, its main weakness was that quantitative analysis of the contaminants and transformation products was subject to interference arising from compounds co-desorbed from the complex biofilm matrix. Only crude separation was attainable and the resulting mass fingerprint was complex as a wide range of analytes underwent electron impact ionization and subsequent fragmentation in the ion source.

The two anticipated advantages of the new procedure over the previous direct probe MS analysis of contaminants [10,19,20] are thus (a) the chromatographic separation of interferences from the compound(s) of interest and (b) use of a soft ionization promoting the formation of abundant $[M+H]^+$ ions, well suited for subsequent MS–MS confirmation of the parent molecule. *N*-Methylpyrrolidinone (NMP) and its primary photo-oxidation intermediate *N*-methylsuccinimide (NMS) (Fig. 1) have been used as model compounds in previous photo-oxidation studies [21], and were therefore used in this study.

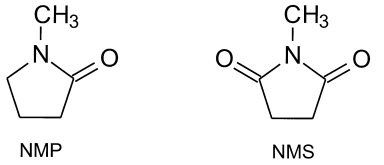

Fig. 1. *N*-Methylpyrrolidinone (NMP) and *N*-methylsuccinimide (NMS).

2. Experimental

2.1. Materials and photooxidation procedure

TiO_2 (P25 grade) was used as obtained from Degussa (Ridgefield Park, NJ, USA). NMP was used as received from Aldrich (Oakville, Canada).

The arrangement for the photocatalytic step (Fig. 2a) consisted of an annular quartz cell (volume 150 ml) which surrounded a 15 W lamp (Philips TUV low-pressure Hg lamp, 254 nm). Suspensions of the TiO_2 photocatalyst (1 g l^{-1}, Degussa P25) and aqueous contaminant solutions (in distilled deionized water) were kept aerated by a steady stream of air. Irradiation periods were 2.5 min, after which the slurry was filtered. The incident light intensity was $7.2(\pm 0.2)\cdot 10^{-6}$ ein s^{-1}, as determined by ferrioxalate actinometry [22,23].

2.2. Bioreactor

A schematic of the bioreactor used in the uptake studies is shown in Fig. 2b. Native Elbe River biofilm was developed in a biofilm reactor which consisted of an outer cylinder with a rotating inner cylinder. Re-circulating tubes in the inner cylinder ensured optimal mixing of the system and minimized nutrient gradients. At the inside of the outer cylinder 12 slides could be withdrawn for monitoring the presence of contaminants in the biofilm at the start and termination of the experiments. For development of mature biofilm, the bioreactor was flooded continuously with Elbe water (flow-rate: 105 ml min^{-1},

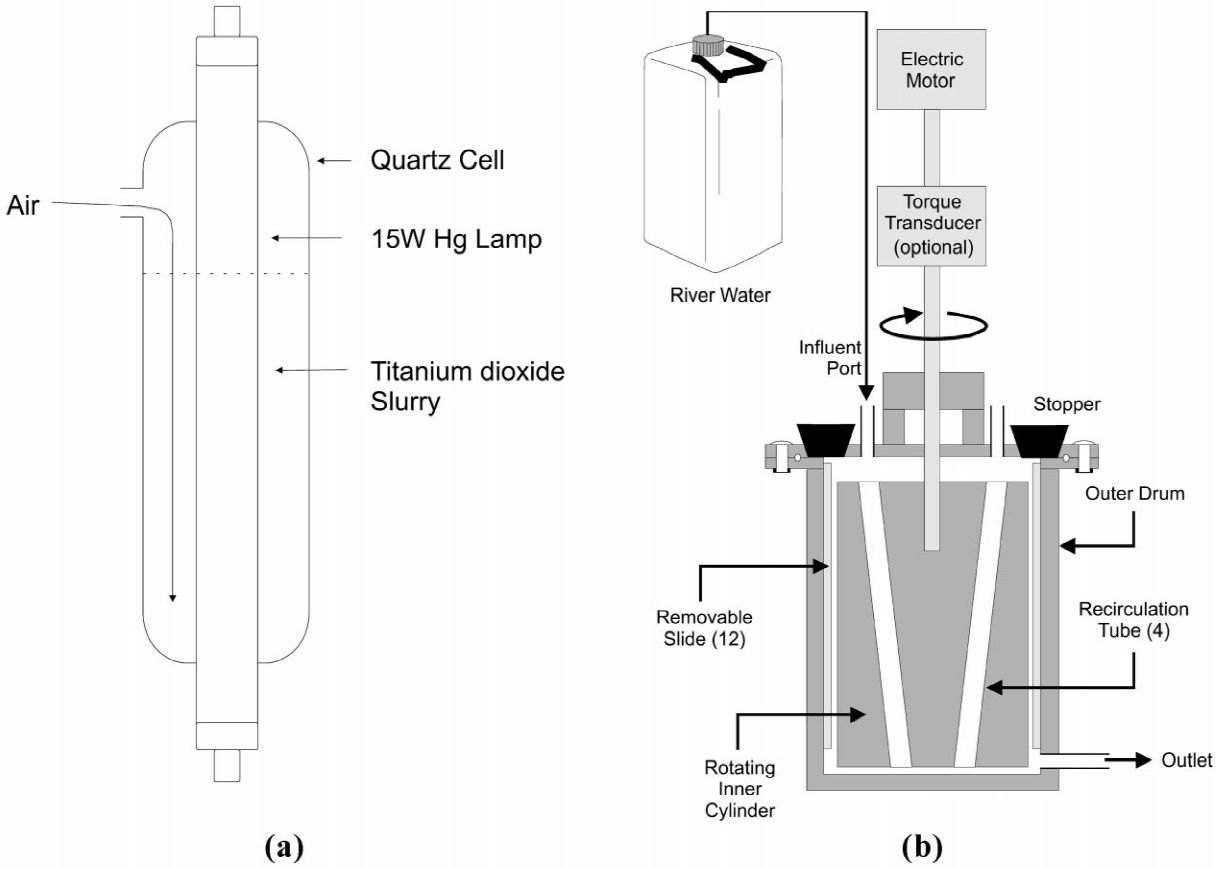

Fig. 2. Schematic diagrams of the (a) photo-oxidation cell, and (b) Roto-Torque bioreactor.

rotation rate: 150 rpm). The biofilm attained a steady state (5–10 mm thickness), after approximately 14 days based on previous measurements of the protein and glucoronic acids in the biofilm.

At the start of the uptake experiment, the flow of Elbe River water was switched off, the water drained through the outlet and the bioreactor filled from the top with 650 ml of the photo-oxidation effluent. After addition, the rotation rate of the reactor was continued at 150 rpm, under zero flow conditions. Thereafter, a time series of the effluent was obtained using triplicate 1 ml samples collected directly into glass vials, which were stored at 4°C until analysis. A biocide, NaN_3, was added before storage. These samples were used for all measurements of sorption to the biofilm by monitoring the concentration of NMP and photo-oxidation intermediates in the effluent using a conventional LC–UV procedure.

In brief, a Waters high-performance liquid chromatography (HPLC) system (Waters, Milford, MA, USA) was used, with a 600E system controller and a Model 484 UV absorbance detector. Water samples were filtered before analysis using a syringe filter with a 0.45-μm membrane (type HA, Millipore, Bedford, MA, USA). This membrane did not measurably adsorb NMP or NMS as determined by LC–UV. A 50-μl volume of the filtered water was injected and eluted isocratically with a mixture of acetonitrile–water (5:95) at 1 ml min^{-1}. Separation was performed using a 15 cm×3.9 mm with 4 μm particle size reversed-phase C_{18} Nova Pak column (Waters). The absorbance detector was set at 212 nm. External calibration was used for the quantification of NMP and NMS. Detection limits under these conditions were approximately 200 μg l^{-1} for NMS and 400 μg l^{-1} for NMP, and the reproducibility was

>95% for both components based on triplicate analyses.

2.3. LC–MS and LC–MS–MS of biofilms

Biofilm samples were ultrasonically extracted with 5 ml of methanol for 1 h at room temperature then filtered using a 0.2 μm pore size surfactant-free cellulose acetate syringe filter (Nalgene, Rochester, NY, USA). The extracts were reduced to 0.5 ml using a dry nitrogen stream and an internal standard, diethanolamine (0.5 μg ml^{-1}) was added to the final extract. A 25 cm×2.1 mm with 5 μm particle size reversed-phase ABZ column (Supelco, Oakville, Canada) was used for the separation [flow-rate=200 μl min^{-1} with a post-column split allowing 30 μl min^{-1} to the electrospray (ES) source] and the samples were eluted isocratically with methanol–water (50:50) (1% formic acid added). The source was cleaned prior to commencing the investigation and cleaned approximately every 40 injections for the duration of the study.

Mass spectrometric analysis was performed using an Autospec Q mass spectrometer (Micromass, Manchester, UK) with an ES interface in the positive ion mode. MS conditions were as follows: source temperature 80°C, cone voltage setting 23 V, ring electrode setting 17 V, needle voltage setting 56.2 V, nebulizer gas (nitrogen) at 15 l h^{-1}, and bath gas nitrogen at 200 l h^{-1}. Resolution was tuned to 1200 and the detector set at 400 V. The ion for NMP, $[M+H]^+$, at m/z 100.1 was collected using selected-ion monitoring (SIM). MS conditions were optimized while infusing a solution of the analytes into the ion source. Loop injections were also performed to optimize sensitivity and ionization while varying solvent composition.

MS–MS confirmation was performed using product ion scans of the $[M+H]^+$ (m/z 100.1) of NMP. MS1 (magnet) was manually parked to allow transmission of m/z 100.1, while MS2 (quadrupole) was scanned from m/z 40–150 at 2 u resolution. Argon was used as the collision gas at a pressure sufficient to attenuate the ion beam by 50% while transmitting the $[M+H]^+$ ion of NMP. Collision energy was set at 17 V (laboratory frame of reference). Under these conditions, the detection limit was 2 ng g^{-1} with a repeatability of 80–95% based on triplicate analyses. NMS was not detected at practical quantitation levels relevant to this study using an Autspec Q with ESI.

Complementary studies were conducted using atmospheric pressure chemical ionization (APCI) in both the positive and negative ion modes. These experiments were performed to explore whether an improved method could be found to overcome the poor sensitivity observed for the intermediate NMS, under ESI. A Micromass Quattro LC (Micromass) triple quadrupole LC–MS–MS mass spectrometer was used for the APCI experiments. Samples (5 μl) were introduced using an HP1100 auto-sampler (Agilent, Palo Alto, CA, USA). A HP1100 LC pump was used to deliver the eluent (water–methanol, 50:50, plus 1% formic acid) at a flow-rate of 250 μl min^{-1}. Chromatographic separation was achieved using the identical column (ABZ, Supelco) as outlined above for ES analysis. The corona voltage was 3.25 kV and the probe temperature 400°C. Cone voltage was optimized at 20 eV to optimize the abundance of $[M+H]^+$. Nitrogen was used as the nebulizer and drying gas at flows of 582 and 414 l h^{-1}, respectively. The data was collected using selected ion recording using the following parameters: interchannel delay 0.02 s, span 0.75 Da, dwell time of 0.08 s for each mass – m/z 100.1 (NMP), m/z 106.1 (DEA the internal standard) and m/z 114.1 (NMS). Under positive ion APCI conditions, the detection limit for NMS in biofilms was 700 ng g^{-1}, a factor of seven times higher than a conventional LC–UV approach. As such high levels are not of environmental relevance, further investigation of NMS in biofilm samples was not pursued by APCI. In comparison, NMP detection limits using APCI were again approximately seven times higher (14 ng g^{-1}) to that obtained using ESI. For APCI experiments, no ions were observed for NMP or NMS under negative ion conditions.

3. Results and discussion

The key findings will be shown to be that although poor sensitivity was observed for the intermediate NMS, the tandem MS technique was well suited for the analysis of NMP in biofilms, confirming that biofilm uptake of the parent compound is effective once biofilm acclimation is achieved. While, there

are implications for photo-oxidation and biofilms in the overall attenuation of aquatic organic contaminants [24–27], focus will be given here to the advantages and limitations of the new chromatographic MS and tandem MS procedure.

Although the detection limits of the conventional LC–UV method were sufficient for the analysis of the bioreactor effluent, (approximately 200 $\mu g\ l^{-1}$ for NMS and approximately 400 $\mu g\ l^{-1}$ for NMP at 212 nm), the LC–UV method was not sufficiently sensitive for the analysis of biofilm extracts (detection limit of NMP 100 ng g^{-1}). This was a direct consequence of the relatively low total amount of biofilm-adsorbed analyte available for analysis, due in part to the limited mass of biofilm that could be collected from the bioreactor. Complementary procedures employing LC–MS and LC–MS–MS were therefore adopted in which the detection limits for NMP were significantly lower (2 ng g^{-1}).

The calibration curve obtained using LC–MS for NMP analysis was curvilinear as illustrated in Fig. 3. Five-point calibration curves were established on a daily basis throughout the course of this study. In all cases the day-to-day calibrations were curvilinear with the respective curves falling within an RSD of ~10%. Such curvilinear calibrations have been reported previously for other analytes using electrospray ionization [28] and appear to be linked to saturation of ions in the ion source at relatively high concentrations of specific compounds [29]. In view that external standards in pure eluent were used to establish the calibration curve, it does not seem likely that ion suppression was due to co-eluting compounds. For some biofilms with co-eluting components, MS–MS was needed to provide added quality assurance and quality control for conclusive confirmation of the analyte. For example, as shown in Fig. 4a, there is a co-eluting peak (at 3.2 min) in the LC chromatogram during NMP analysis. Such co-eluting components, however, did not pose any detectable interference in the product-ion scans used for confirmation of the analyte and thus further chromatographic separation was not pursued. As a confirmation tool, MS–MS of $[M+H]^+$ (m/z 100.1) was obtained (Fig. 4b) and compared to the authentic standard. Good agreement was found, thus confirming the uptake of NMP in the biofilm. In our investigation, additional confirmation using tandem MS was required for 10 to 20% of all runs.

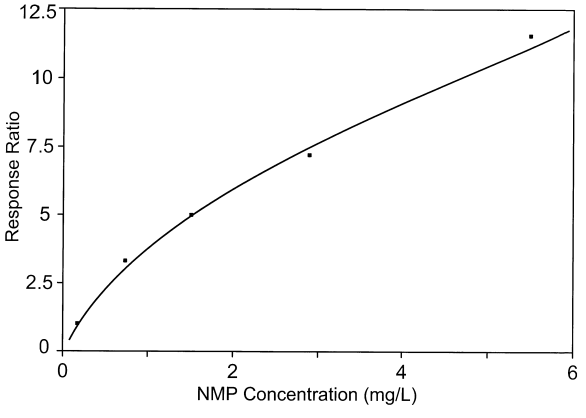

Fig. 3. (a) Example of LC–MS calibration curve of NMP in biofilm matrix.

As a direct consequence of the use of the soft electrospray ionization coupled with the observation that there were little or no interfering ions in the biofilm samples; the application of LC–MS alone could be used for routine quantification of NMP. As expected, chromatography prior to MS analysis facilitated a reduction in possible matrix and salt effects compared to the earlier direct probe procedure [10]. For the latter, the matrix introduced into the mass spectrometer was the riverine biofilm itself. This had the potential of causing the gradual coating of the inside of the source with contaminants and biofilm constituents. With prior extraction of the biofilm with an organic solvent, salts and particulates were also excluded prior to MS analyses, providing an extract that could be preconcentrated for a more representative biofilm sample.

4. Conclusions

The LC–MS and LC–MS–MS procedure was used to quantify NMP in riverine biofilms following photo-oxidation in natural waters, and deionized water. The tandem MS experiments provided confirmation of the presence of the parent compound within the biofilms following an acclimatization delay of about 1 day. A drawback to the LC–MS and LC–MS–MS procedure, however, is that it is biased to compounds which produce ions under electrospray

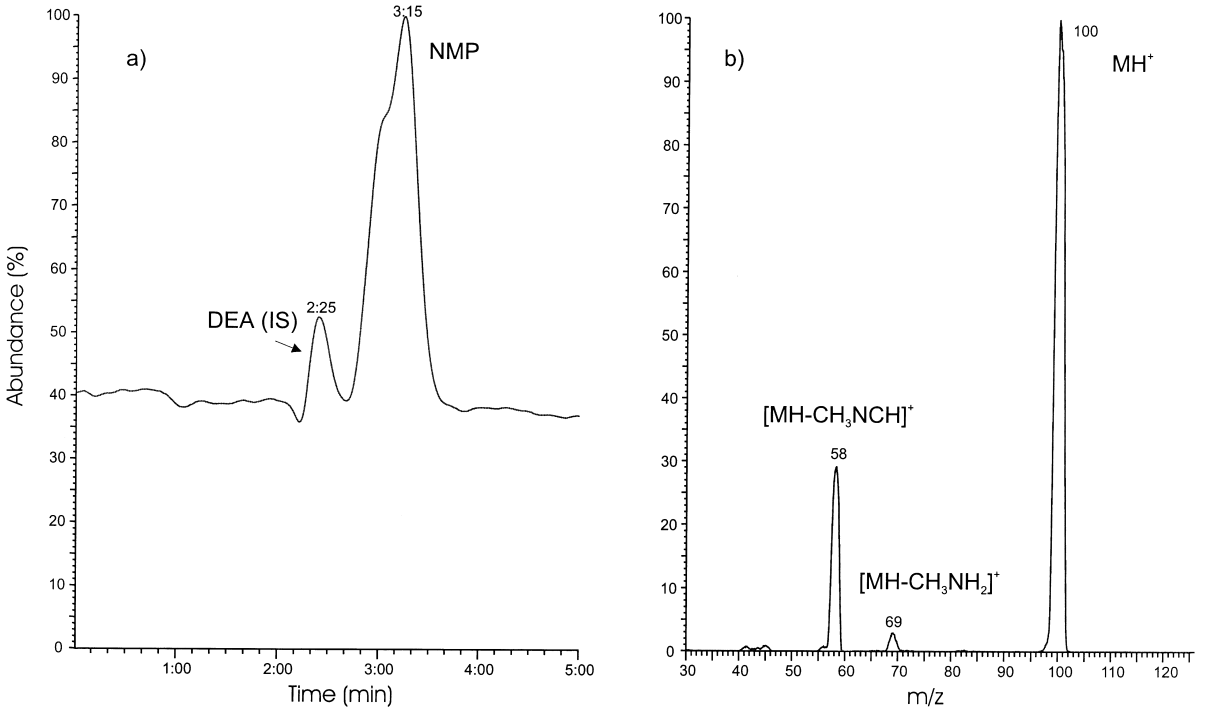

Fig. 4. (a) LC–MS (SIM) chromatogram of NMP (peak at 3.2 min) in biofilm extract. (b) MS–MS spectrum of $[M+H]^+$ ion (m/z 100.1). Conditions as outlined in text.

ionization. In this study, it meant that although NMP could be detected in the biofilm, NMS could not. This intermediate was also not well suited to analyses using atmospheric pressure chemical ionization. Alternate ionization modes and/or chemical manipulations of the analyte may thus be required to widen the scope of detectable compounds for some applications.

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References

[1] T.R. Neu, J.R. Lawrence, FEMS Microbiol. Ecol. 24 (1997) 11.
[2] B.E. Christensen, W.G. Characklis, in: W.G. Characklis, K.C. Marshall (Eds.), Biofilms, Wiley, New York, 1990, p. 55.
[3] W.F. Dudman, in: I.W. Sutherland (Ed.), Surface Carbohydrates of the Prokaryotic Cell, Academic Press, London, 1977, p. 357.
[4] Y. Tago, K. Aida, Appl. Environ. Microbiol. 34 (1977) 308.
[5] J.W. Costerton, R.T. Irvin, K.J. Cheng, Annu. Rev. Microbiol. 35 (1981) 299.
[6] J.W. Costerton, Dev. Ind. Microbiol. 25 (1984) 363.
[7] A.W. Decho, Oceanogr. Mar. Biol. Annu. Rev. 28 (1990) 73.
[8] L.K. Nguyen, N.L. Schiller, Curr. Microbiol. 18 (1989) 323.
[9] J.V. Headley, J. Gandrass, J. Kuballa, K.M. Peru, Y. Gong, Environ. Sci. Technol. 32 (1998) 3968.
[10] J.V. Headley, K.M. Peru, J.R. Lawrence, G.M. Wolfaardt, Anal. Chem. 67 (1995) 1831.
[11] G.M. Wolfaardt, J.R. Lawrence, R.D. Robarts, D.E. Caldwell, Appl. Environ. Microbiol. 61 (1995) 152.
[12] G.M. Wolfaardt, J.R. Lawrence, J.V. Headley, R.D. Robarts, D.E. Caldwell, Microb. Ecol. 27 (1994) 279.
[13] T. Rudd, R.M. Sterritt, J.N. Lester, Microb. Ecol. 9 (1983) 261.
[14] G. Geesey, L. Jang, in: H.C. Ehrlich, C.L. Brierley (Eds.), Microbial Mineral Recovery, McGraw-Hill, New York, 1990, p. 223.
[15] C. Freeman, P.J. Chapman, K. Gilman, B. Reynolds, H.S. Wheater, Hydrobiologia 297 (1995) 61.
[16] J. Sketchell, H.G. Peterson, N. Christofi, Water Qual. Res. J. Canada 34 (1999) 615.

[17] M.R. Hoffman, S.T. Martin, W. Choi, D.W. Bahnemann, Chem. Rev. 95 (1995) 69.
[18] D.F. Ollis, H. Al-Ekabi (Eds.), Photocatalytic Purification and Treatment of Water and Air, Elsevier, Amsterdam, 1993.
[19] G. Kopf, J.V. Headley, K.M. Peru, R.C. Johnson, M.H. Soni, R.G. Cooks, J. Rapid Commun. Mass Spectrom. 11 (1997) 24.
[20] J.V. Headley, K.M. Peru, M.T. Arts, Anal. Chem. 67 (1995) 4349.
[21] D.A. Friesen, J.V. Headley, C.H. Langford, Environ. Sci. Technol. 33 (1999) 3193.
[22] C.G. Hatchard, C.A. Parker, Proc. R. Soc. A 235 (1956) 518.
[23] A.D. Kirk, C. Namasivayam, Anal. Chem. 55 (1983) 2428.
[24] R.A. Sierka, C.W. Bryant, Water Sci. Technol. 29 (1994) 209.
[25] S. Tanaka, T. Ichikawa, Water Sci. Technol. 28 (1993) 103.
[26] V.B. Manilal, A. Haridas, R. Alexander, G.D. Surender, Water Res. 26 (1992) 1035.
[27] J.P. Scott, D.F. Ollis, Environ. Prog. 14 (1995) 88.
[28] B. Køppen, N.H. Spliid, J. Chromatogr. A 736 (1996) 105.
[29] T.L. Constantopoulous, G.S. Jackson, C.G. Enke, Anal. Chim. Acta 406 (2000) 37.