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Recalcitrance of poly(vinylpyrrolidone): evidence through matrix-assisted laser desorption–ionization time-of-flight mass spectrometry

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Abstract

The aerobic biodegradability of an extensively used synthetic polymer was monitored the first time on a laboratory-scale fixed-bed bioreactor (FBBR) applying matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI-TOF MS). Polymeric poly(vinylpyrrolidone) (PVP) was spiked at concentrations of 10 mg l^{-1} onto the FBBR run with river water and the biodegradation monitored after lyophilization of aliquots of the test liquor applying MALDI-TOF-MS. The latter proved to be a powerful tool for qualitative screening purposes of PVP in a molecular mass range <20 kDa in particularly yielding a high sensitivity and shot-to-shot reproducibility. The sample-to-sample reproducibility was enhanced applying the anchor target device. Post-source decay-MALDI-TOF-MS fragmentation investigations determined the unknown end groups of PVP unambiguously. Poor biodegradability of PVP can be assumed, since even after 30 days, no oxidation of the terminal groups and no difference in the repeating units was observed. A decrease in the molecular mass distribution can be drawn back rather to adsorption of PVP in the FBBR other than to biodegradation. This was further investigated performing an adsorption experiment with sewage sludge as solid matrix and analyses of the aqueous phase and sludge samples. Extrapolating these results to the situation in wastewater treatment plants, it is highly likely that PVP is eliminated from the dissolved phase by adsorption onto sludge particles.

Keywords: Matrix-assisted laser desorption-ionization time-of-flight mass spectrometry; Environment; Biodegradation; Poly(vinylpyrrolidone)

1. Introduction

1.1. Production and use

Poly-*N*-vinylamides such as poly(vinylpyrrolidone) (PVP, Fig. 1) are highly water soluble synthetic polymers with a weighted average molecular weight (M_w) between 2.5 and 1200 kDa and broad M_w distribution. These main physiochemical parameters distinguish the area of their applications. Soluble

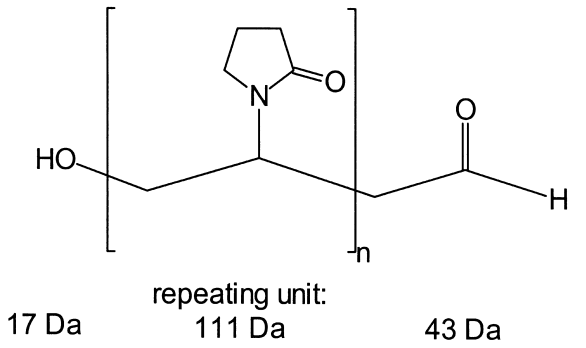

Fig. 1. Chemical structure of PVP 2.5kDa (n=4–50).

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PVP of almost any M_w is obtained by free-radical polymerization of vinylpyrrolidone in water or isopropanol [1].

The most beneficial chemical property of PVP is its interaction with low- and high-M_w compounds in aqueous solutions such as inorganic anions, organic compounds (e.g. azodyes, phenols), amino acids, surface active substances, polymers, proteins and pharmacologically active substances [2]. This property is especially significant in developing medicinal preparations. The different M_w materials are distinguished by the K-number such as PVP K12, K17, K25 and K30, which have their applicability in pharmaceutical, cosmetic and food industries, for example to enhance solubility and transportation properties in tablets.

Soluble PVP was first used during World War II as a blood-plasma substitute, but while it is not metabolized, small quantities of high M_w components may be retained within the body after intravenous administration. Therefore, today its use for this purpose is restricted to oral administration. Numerous studies concerning excretion and metabolism of PVP after intravenous administration established that the rate and the extent of clearance of PVP macromolecules through kidneys is dependent on molecular size [3,4].

Today, soluble PVP (e.g. Kollidon, Polyvidon(e), Povidone, Plasdone) is one of the most versatile and widely used pharmaceutical auxiliaries. It is also used in the production of one of the most important topical disinfectants, PVP–iodine. Another important field of application is the use of homomeric and copolymeric PVP (10–60 kDa with polydispersities >10) as dye transfer inhibitor [5,6] in the formulation of laundry detergents.

1.2. Analysis and environmental fate

From the perspective of PVP in the aquatic environment after release from extensive domestic and industrial use, its biodegradation and fate is poorly investigated. This lack is due to challenging analytical methods required for the investigation of this new class of target analyte that PVP represents: a water soluble polymer with various molecular mass distributions above 2 kDa.

In order to address the fate of environmentally relevant compounds, investigations to be carried out should enable the qualitative and quantitative characterization of the analyte and possible metabolites. In previous works the primary biodegradation of a broad variety of chemically different pollutants, such as e.g. pesticides, surfactants, chelating agents and flame reductants has been studied on a laboratory-scale fixed-bed bioreactor (FBBR) in combination with either a liquid chromatography–electrospray–mass spectrometry (LC–ESI-MS) or gas chromatography (GC)–MS detection method and thereby proved to be a good model system for such purposes [7,8–11]. But for monitoring the occurrence of PVP it would not be applicable in this combination since commercially used samples of PVP with the lowest M_w exceed the mass range of most commercial mass spectrometric methods.

Identification methods for PVP described in the literature are e.g. infrared spectroscopy for the qualitative analysis, photometry of the PVP–iodine complex [12,13] or pyrolytic GC [14,15] for the quantitative analysis of PVP, whereas light scattering [16,17], sedimentation [18] and gel-permeation chromatography (GPC) [19] are commonly applied for the determination of the M_w.

Since all these approaches are not applicable for the detection of PVP at a low mg l^{-1} level and in a complex environmental matrix such as wastewater or sewage sludge, a matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI-TOF-MS) analysis was taken into consideration as an extension of a detection method for FBBR investigations and to monitor environmental samples.

MALDI-TOF-MS has been shown in the last decade to be a powerful tool for the analysis of a wide range of biological and synthetic macromolecules with no serious upper M_w limitations (>1 million Da) [20]. Additional advantages are the ''soft'' ionization–desorption process that allows for the detection of intact soluble [20] and insoluble [21] macromolecules, the short analysis time for routine analysis and high accuracy. Hence, the qualitative MALDI-TOF-MS analysis of a large variety of analytes has been established for more than 10 years in very different fields of analytical chemistry but showed poor performance regarding quantitative investigations. Thus, successful MALDI-TOF-MS quantification was only obtainable in special cases under particular circumstances [22]. In order to determine the fate of PVP during biodegradation and

adsorption study to sludge, our methodological approach was to apply (i) the conventional sample preparation method for MALDI-TOF-MS for qualitative screening purposes, (ii) the anchor target as promising device for quantification experiments and (iii) post source decay (PSD)-MALDI-TOF-MS for unequivocal end group analysis.

2. Experimental

2.1. Materials

2.1.1. Analytes

PVP 2.5 kDa (pharmaceutical purity: residual monomer $<0.1\%$, ash $<0.02\%$, heavy metals <10 mg kg^{-1}, aldehydes $<0.1–0.2\%$, hydrazine <2 mg kg^{-1}) were from Polysciences Europe (Eppelheim, Germany). PVP 22.1 (*D* 7.37) and 14.4 kDa (*D* 10.87) and polystyrene (PS) 2 kDa were obtained from PSS (Mainz, Germany). PVP K12 (2–3 kDa), K17 (7–11 kDa) and K25 (28–34 kDa) were placed at our disposal by BASF (Ludwigshafen, Germany). PVP K25 and K30 were purchased from Aldrich (Steinheim, Germany).

2.1.2. Matrices

3β-Indole acrylic acid (IAA) and 2,5-dihydroxy benzoic acid (DHB) were obtained from Aldrich. α-Cyanohydroxy cinnamic acid (HCCA) was from Sigma (St. Louis, MO, USA).

2.1.3. Metal salts

Silver trifluoroacetate, lithium trifluoroacetate, sodium trifluoroacetate, potassium trifluoroacetate were purchased from Aldrich and used without further purification.

2.1.4. Solvents

The employed solvent methanol (99.9%) was obtained from Riedel-de Haen (Seelze, Germany). Tetrahydrofuran (p.a.) (THF) was obtained from Fluka (Buchs, Switzerland). Acetonitrile and formaldehyde (p.a.) were from Merck (Darmstadt, Germany).

2.2. Laboratory-scale fixed-bed bioreactor set-up

The set-up of the FBBR is described in detail in a previous work [7]. Briefly, river Rhine water is pumped in circuit over a fixed-bed made up of porous glass beads. The bulk solution in the storage tank is aerated via a glass frit to guarantee aerobic conditions in the system.

2.3. Biodegradation experiment

River Rhine water was supplemented with PVP 2.5 kDa at a concentration of 10 mg l^{-1}. Over a period of 30 days, 20-ml samples were taken every 2 days except for the first day where sampling was done at shorter intervals. The samples were immediately preserved by addition of 1 ml formaldehyde. For MALDI-TOF-MS measurements 10 ml of each sample were lyophilized, reconstituted with 1 ml water–acetonitrile (95:5, v/v) and 0.45-μm filtrated.

2.4. Adsorption experiment

Anaerobically digested and dewatered sludge from a municipal wastewater treatment plant was lyophilized and the recovered dry sludge ground with the aid of a mortar. Of this, 250 mg were suspended in 5 ml water in a 10-ml centrifuge tube. An aqueous solution of PVP with an average M_w of 6 kDa, obtained from PVP K17 fractionated by GPC (Fig. 2), was used for spiking to yield a final concentration of 100 mg l^{-1}. Two control samples, one containing no PVP spike, the other no sludge, were prepared likewise. The tubes were then shaken horizontally for 1 day. Samples of 100 μl volume were taken from the supernatant after centrifugation for 15 min at 3000 rpm and submitted to the MALDI sample preparation.

2.5. Gel-permeation chromatography

All PVP samples with a M_w above 5 kDa were fractionated by GPC. The samples were dissolved in deionized water. The extraction was obtained by 0.1 M $NaNO_3$ in 20% acetonitrile.

2.6. Sample preparation for MALDI-TOF-MS

2.6.1. Conventional MALDI-TOF-MS sample preparation

The conventional MALDI target with 26 spots from Bruker (Bremen, Germany) was applied. The

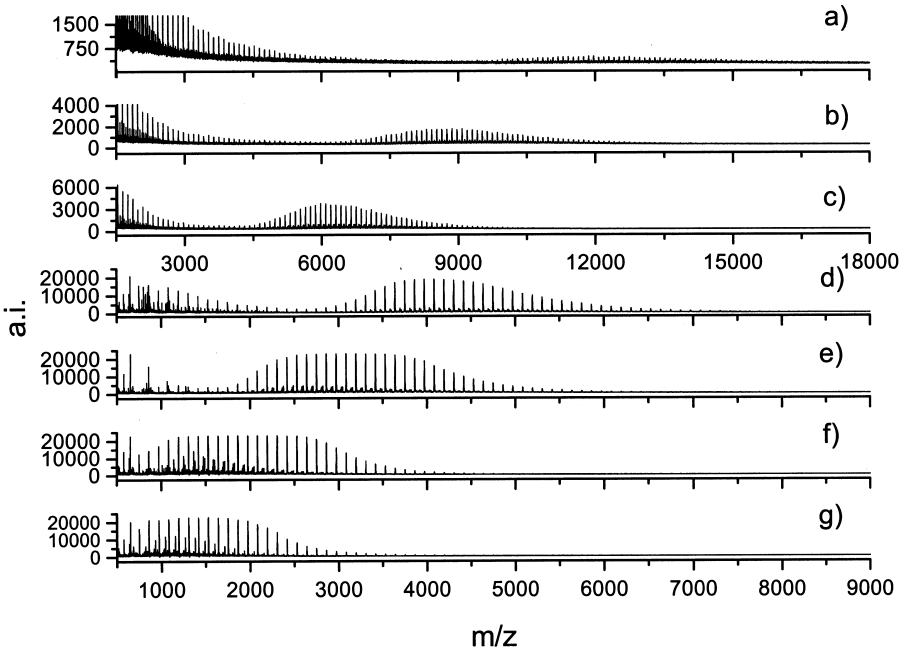

Fig. 2. MALDI-TOF mass spectra of the GPC fractions of PVP K17: (a) fraction 1, (b) fraction 2, (c) fraction 3, (d) fraction 4, (e) fraction 5, (f) fraction 6, (g) fraction 7.

optimal concentration for the degradation study was found to be 7.5 g l^{-1} (in methanol) of the MALDI matrix 3β-indole acrylic acid (IAA) and 3 g l^{-1} (in methanol) of the cationization agent sodium trifluoroacetate (Na salt). A 10-μl volume of the IAA solution, 10 μl of the aqueous PVP sample and 1 μl of the salt solution were added together, mixed and applied to the MALDI target (1 μl). The measurements were carried out immediately after evaporation of the solvent. The different metal salt solutions such as Li, K, Ag were prepared at the same concentrations as the Na salt, and the sample preparation was carried out analogously.

2.6.2. MALDI-TOF-MS sample preparation with the anchor target device

An anchor target chip device™ (Bruker Daltonik, Bremen, Germany) consists of a hydrophobic surface and several hydrophilic spots where the sample has to be deposited. For the MALDI-TOF-MS measurements with the anchor target device (600 μm diameter, 36 spots) the salt amount was lowered such that 0.5 μl was added to 10 μl the IAA solution and 10 μl of the aqueous PVP sample. A 0.1-μl volume of the homogeneous MALDI sample solution was applied to the target spot.

2.7. Mass spectrometry

2.7.1. MALDI-TOF-MS instrument

MALDI-TOF mass spectra were recorded using a Bruker Reflex II™ MALDI-TOF mass spectrometer equipped with a scout 26 and a N_2-laser ($\lambda = 337$ nm) operating at a pulse rate of 3 Hz. The ions were accelerated with pulsed ion extraction (PIE™ design from Bruker Daltonik) by a voltage of 20 kV. The analyzer was operated in reflection mode and the ions were detected using a microchannel plate detector.

2.7.2. Calibration and experimental conditions in MALDI

Calibration of the instrument was carried out before each measurement using PS (2 kDa) dissolved in THF together with dithranol as matrix and silver salt (1:500:10). Several isotopic resolved PS-oligomers were used for a multipoint calibration.

The signal intensity of PVP depended strongly on

the applied laser power and increased rapidly at higher values, whereas mass resolution decreased. To reinsure the same experimental conditions for the different samples (e.g. laser power, extraction voltage, detection voltage) these parameters were kept unchanged.

3. Results and discussion

Since MALDI-TOF-MS is not only the first time tested for the qualitative characterization of PVP itself, but further exploited as a detection method for biodegradation studies of polymeric wastewater relevant compounds with broad polydispersities, several investigations needed to be performed. Thus, MALDI-TOF-MS had first to be evaluated for the upper M_w limitations for the characterization of PVP and then tested for its screening capacity in low concentrations for unequivocal characterization in surface and wastewater according to its sensitivity, reproducibility, homogeneity and quantification potential. Hence, keeping a certain level of reproducibility, monitoring of the degradation can be perceptible either by deformation and shift to lower oligomers of the polymer distribution and/or by the appearance of a new polymer distribution, e.g. through oxidation of a terminal group.

3.1. MALDI-TOF-MS analysis for biodegradation studies

The main purpose of these preliminary experiments included the evaluation of the upper M_w limitations for the characterization of PVP and the investigation of an experimental set-up for monitoring the biodegradation of this analyte and possible metabolites.

Although qualitative MALDI-TOF-MS investigations of PVP K17 (7–11 kDa) gave the mass of the repeating units (111 Da, Fig. 1), the M_w distribution was strongly shifted to low mass oligomers in a mass region of 1–5 kDa (data not shown).The suppression of higher M_w components resulting in an overestimated detection of low M_w oligomers originates from the crucial point of high polydispersity (D) of >3 kDa, which is known to govern the M_w distribution in MALDI-TOF-MS analysis in general [23,24]. Thus, samples with high values of D have to be fractionated by GPC before MALDI-TOF analysis to lower the polydispersity of the sample. GPC fractionation of PVP K17 made it accessible to mass spectrometric characterization and the M_w of the fractions determined by MALDI-TOF-MS ranged from <1 kDa to about 17 kDa (Fig. 2a–g).

In each PVP sample small amounts of low M_w oligomers of GPC-fractionated PVP samples (e.g. Fig. 2) were detected in MALDI-TOF-MS to such a high extent that finally, in particularly with increasing M_w of the investigated samples, the detection of the fractions itself was prevented by low M_w oligomers. For example, the analysis of PVP K25 (28–34 kDa) allowed to detect besides the abundant low M_w oligomers ($\sim$ 1–5 kDa) a M_w distribution of the individual GPC-fractions only up to 15 kDa (data not shown). The relatively too low M_w range can be most likely attributed to suppression effects resulting from the lower oligomers.

The applied methodological approach furthermore allowed the successful characterization of PVP 14 kDa (D 10.87), but only to some extent the analysis of PVP 22.1 kDa (D 7.37), K25 and K30 (data not shown). Hence, the combination of the GPC and the MALDI-TOF-MS method appeared to have limitations for the higher M_w analysis of PVP (<20 kDa) but still allowed to partially cover the characterization of PVP with various molecular masses of the industrial application spectrum (e.g. pharmaceutical, cosmetic and food industry) and thus appeared to be impractical for FBBR degradation and quantification purposes of PVP presented herein.

Therefore a low M_w PVP 2.5 kDa was taken for the FBBR biodegradation investigations. This analyte mass region appeared to be valuable since it showed a sufficient span to pursue the degradation of the analyte while not forfeiting a quality decrease in the mass spectra due to interference of background and matrix-adduct-formation commonly observed in the mass region <1 kDa [25]. The additional advantage of this mass region is the obtainable isotopic mass resolution and therefore an increased accuracy.

Since PVP dissolves in high concentration only in water [26], the choice of MALDI matrices was limited to water compatible matrices such as IAA, HCCA and DHB. From these only IAA gave ap-

preciable homogeneity, whereas the others showed stronger sample inhomogeneity and poor quality in the mass spectra (data not shown).

Polymeric analytes commonly depend on cationizing agents other than proton sources to allow for successful ionization and detection in mass spectrometry. The stable formation of pseudo-molecular-ions is governed by different effects such as polymer length, cation size, structure of the analyte (e.g. heteroatoms, aromatic rings, end groups) [27,28]. When no metal salt was added, a mixture of different adduct ions was detected whereas the most intense signal appeared to be from a sodiated polymer chain. The MALDI-TOF mass spectra of PVP showed a quality increase with any metal salt added and proved to form the most stable adduct ions with sodium yielding the most intense signals (Fig. 3a). The mass spectra of the lithiatium cationized PVP (Fig. 3c) showed an overestimation of the lower oligomers. Regarding signal intensity and quality in the mass spectra following decreasing order with respect to cationizing agents (see Fig. 3) was found for PVP: sodium>lithium>potassium>silver>no metal cation added. With these changes in metal salts, the unknown end groups could be calculated as a sum mass of 60 Da equivalent to $C_2H_4O_2$. This is in correlation with the original synthetic path of free radical polymerization of vinylpyrrolidone in water using hydrogen peroxide as initiator leading to end groups such as a hydroxy (HO−) and an aldehyde function (–CH_2CHO) (Fig. 1). The results for the end group determination were verified with post source decay (PSD)-MALDI-TOF fragmentation investigations of a lithium cationized PVP adduct against standard samples of PVP K12 with the known end groups [1,2] as mentioned above (data not shown).

The influence of laser power showed to be a very sensitive parameter that affected the resolution and signal intensity. The latter even in favor of strongly competing signals (Fig. 4). The mass spectrum utilizing low laser power (Fig. 4a) showed the most intense signal series with a repeating unit of 111 Da (=vinylpyrrolidone). This signal at −38 Da relative to the main signal series (Fig. 4b and c) which corresponds to the evaluated structure (Fig. 1) could not be rationalized by assumed end groups neither to any pseudo-molecular ion polymer adduct formation with any common metal salt. Hence, this to us unknown PVP structure can be most likely attributed to PVP with an unknown end group functionality. With increasing laser power the signal intensity for the PVP adduct with an unknown end group functionality decreased (Fig. 4b; medium laser power Fig. 4c; high laser power) whereas the signal intensity for the sodium cationized PVP adduct with an known end group functionality increased. This effect is so strong that the ratios of the signal intensities for the most intense signals reversed (Fig. 4a and c). However, at high laser power the baseline shifted

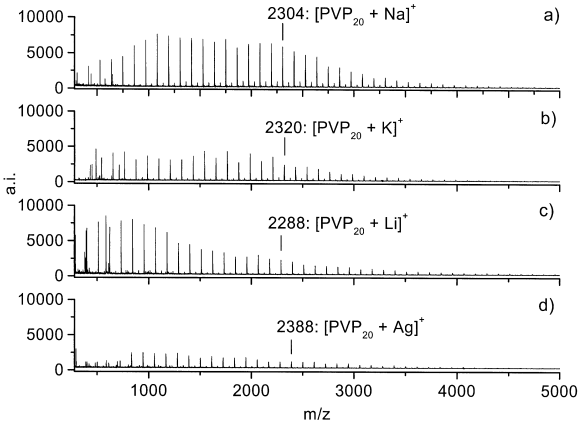

Fig. 3. MALDI-TOF mass spectra of PVP 2.5 kDa in dependence of the added metal salts: (a) sodium, (b) potassium, (c) lithium, (d) silver.

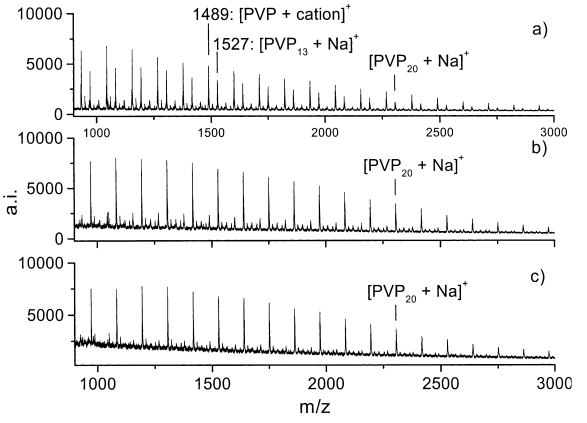

Fig. 4. MALDI-TOF mass spectra of PVP 2.5 kDa (+Na) in dependence of the added laser power: (a) low laser power, (b) medium laser power, (c) high laser power.

and therefore the laser power was kept on a medium level for further investigations of PVP.

PVP spiked to both, demineralized water (data not shown) and river Rhine water (Fig. 5a–c) at concentrations of 1000, 100 and 10 mg l^{-1} confirmed the independence with respect to the water matrix chosen. This is a remarkable as well as a valuable result as MALDI-TOF-MS appears as a very robust and fast method that prevents from time-consuming cleaning and/or desalting processes for the direct analysis of natural water samples with respect to PVP analysis. Hence, MALDI-TOF-MS indicates to be a powerful method for direct screening purposes even for analytes in a molecular mass range where other methods completely fail. The strongest drawback is the non linearity between signal intensity in the MALDI-TOF mass spectrum and the concentration of PVP. Neither in the applied concentration series with demineralized water nor with river water there was a 10-fold decrease of the signal intensity reflected in the MALDI-TOF mass spectra (Fig. 5a–c). The concentration of 1 mg l^{-1} gave irreproducible poor mass spectra with very low signal intensities (data not shown). Therefore a concentration of 10 mg l^{-1} PVP in river water was chosen as starting value to monitor a possible biodegradation, since lower concentrations allow to quantify more accurate even though the background and matrix-adduct-formation had a stronger impact on the mass spectrum. Additionally, it is also important

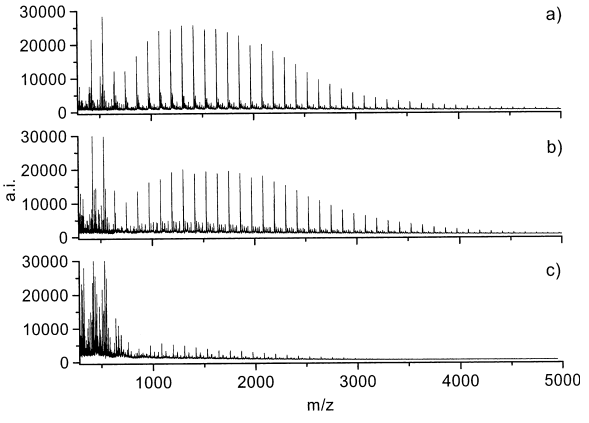

Fig. 5. MALDI-TOF mass spectra of PVP 2.5 kDa (+Na) in dependence of the concentration: (a) 1000 mg l^{-1}, (b) 100 mg l^{-1}, (c) 10 mg l^{-1}.

to note that the decrease in concentration was visible in the MALDI-TOF mass spectrum by a decrease in signal intensity (Fig. 5a–c) that particularly prevents the detection of higher M_w oligomers. A shift in the M_w distribution was suggested in particularly lowering the concentrations (Fig. 5b and c). This seriously interfered with a straightforward interpretation of monitoring the biodegradation step by a shift in the M_w distribution. Consequently, only a strong deformation and decrease in the M_w distribution of the polymer or the formation of a bimodale distribution will indicate unambiguously a biodegradation.

The reproducibility of the MALDI-TOF measurements was tested and repeated by a slot of 2×10 analogous MALDI-TOF-MS sample preparations of PVP (10 mg l^{-1}). A high shot-to-shot reproducibility was found on each spot whereas the sample-to-sample reproducibility from one to another spot was limited (data not shown). The reproducibility even seemed to be independent of the water matrix since demineralized water and river water gave similar results. The overall reproducibility of the PVP analysis indicated to be sufficient for FBBR biodegradation experiments but as expected not for accurate quantification purposes.

Several reasons partly explain the lack of a general, quantitative MALDI-TOF-MS. Since MALDI-TOF-MS is a pulsed laser method, there is neither a quantitative correlation between the amount of sample that is desorbed–ionized per pulse nor a quantitative correlation between the laser power and signal intensity in the mass spectrum. Additionally, there is no reliable information to the size and thickness of the layer of the crystallized matrix/analyte MALDI sample mixture. Thus, signal intensities in MALDI-TOF-MS can vary much stronger than with solvent-based methods in mass spectrometry such as LC–ESI-MS. Despite of all these reservations, the mass range of this polymeric target analyte is very restrictive, hence, MALDI-TOF-MS is the most promising analytical tool for a qualitative and a semiquantitative analysis of this kind of analyte.

For the suitability of the applied method to follow up a concentrations decrease during the biodegradation experiment below 1 mg l^{-1} and eventually analyze real samples, solid-phase extraction (SPE) and lyophilization were compared for their suitability

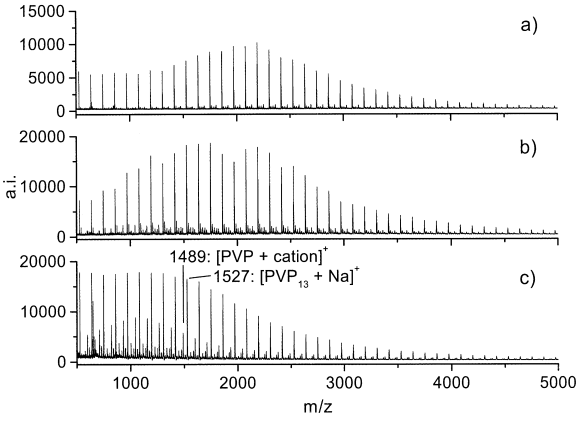

Fig. 6. MALDI-TOF mass spectra of PVP 2.5 kDa (+Na) of enrichment procedures: (a) PVP spiked to river water (100 mg l^{-1}), (b) lyophilization of PVP spiked river water (10 mg l^{-1}), (c) solid-phase extraction of PVP spiked river water (10 mg l^{-1}).

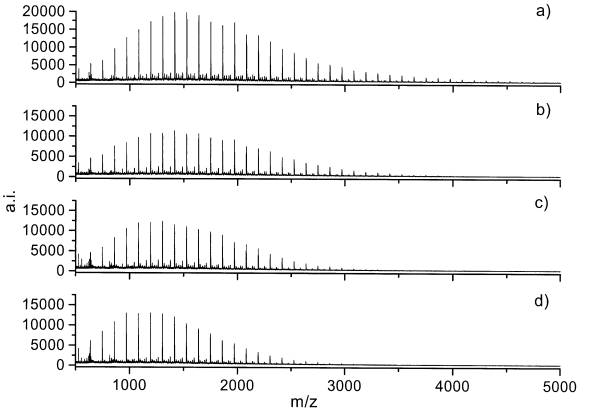

Fig. 7. MALDI-TOF mass spectra of the FBBR degradation course of PVP 2.5 kDa (+Na): (a) 0 h, (b) 5 h, (c) 8 days, (d) 16 days.

of the tenfold enrichment of PVP from surface water. Fig. 6a shows the mass spectrum of PVP spiked to river water. It turned out that the mass spectrum of the SPE enrichment procedure (Fig. 6c) showed an overrepresentation of the lower oligomers and an accumulation of the PVP oligomer with unknown end groups to a fair extent (see also Fig. 4) whereas lyophilization of the PVP spiked river water (Fig. 6b) gave reproducible results unaffecting the M_w distribution severely.

3.2. Monitoring the fate of PVP

The follow-up of PVP, spiked at a concentration of 10 mg l^{-1} onto the FBBR over a period of 30 days, there was neither a collapse in the analyte distribution, usually observed in biodegradation experiments after a certain time of acclimation, nor a rising distribution of a potential metabolite indicating the recalcitrance of PVP (Fig. 7).

A slow diminishing of higher M_w oligomers during the course of the FBBR experiment (Fig. 7a–d) can be explained by a decrease in concentration which involves a decrease in signal intensity. This slowly vanishing process very likely does not indicate a biodegradation, since the signal intensities of the smaller M_w oligomers in the polymer distribution were about identical after 1 day (data not shown) for the whole period of time (Fig. 7c and d) and only varied in the error of reproducibility. It rather can be attributed to an adsorption phenomena, in particular of higher M_w oligomers of PVP to interact and remain to the interior in the FBBR gadget, most likely on the fixed-bed material made up of porous glass beads.

The FBBR degradation results were verified repeating the MALDI-TOF-MS measurements of the complete biodegradation experiment. The same tendency was observed and a misinterpretation due to an error of reproducibility in MALDI-TOF-MS could be excluded. The FBBR experiment indicated a slow, insignificant concentration decrease.

A verification of the recalcitrance of PVP against microbial degradation and its strong adsorption tendency was obtained by an experiment performed in sludge suspensions and monitored by MALDI-TOF-MS. Dry sewage sludge was mixed with an aqueous PVP solution of 100 mg l^{-1} in a mass ratio of 500:1 and well shaken.

The PVP sample was chosen using the following criteria: high M_w oligomers with a low extent of small oligomeric compounds. Hence, the main GPC-fraction of K17 (sodium salt added) was taken, showing its main M_w distribution with good signal intensities from about 5–8 kDa in the MALDI-TOF mass spectrum (comparable to Fig. 2c). The adsorption of PVP to the sewage sludge was verified by

analyzing aliquots of the aqueous supernatant by investigating the decrease in the mother liquor with the time of incubation.

The sensitivity of conventional MALDI-TOF-MS was insufficient to detect the PVP even after a short period of time of incubation with sewage sludge (1 h; data not shown). Such a significant process asked for high sensitivity of the detection step. This was realized by MALDI-TOF-MS investigations using an anchor target chip device. The evaluation of the optimal concentration for the MALDI anchor target device showed about a 10-fold increase in sensitivity compared with the conventional MALDI target. The MALDI-TOF-MS measurements with the anchor target device of the adsorption experiment were indeed able to detect PVP (2–4 kDa mass region) in the aqueous mother liquor after an incubation period of 1 h (Fig. 8a) and failed after 2.5 h (Fig. 8b), too. Thus, these MALDI-TOF-MS measurements suggested that the adsorption of PVP to the sewage sludge appears to be a very fast process and that the PVP concentration in the mother liquor was below 10 mg l^{-1} after 1 h and below 1 mg l^{-1} after 2.5 h. Additionally, the adsorption process of this PVP sample proves to be a size-dependent process because only the lower oligomers where detectable after 1 h (Fig. 8a) whereas the higher components of the used GPC-fraction disappeared completely. These findings are in strong correlation with the FBBR experiment itself (Fig. 7). The control experiment run parallel showed that PVP dissolved in water without the presence of sludge did not show any decrease in the PVP concentration in the mother liquor. This is exemplary shown in Fig. 8c and d (1 h, 32 h) for MALDI-TOF mass spectra obtained with the anchor target device. The results of the adsorption together with the control experiment confirm unambiguously that the PVP is absorbed to the sludge rapidly and to a very high extent.

In any MALDI-TOF-MS measurement of the mother liquor of the PVP adsorption experiment there was an additional polymer distribution ob-

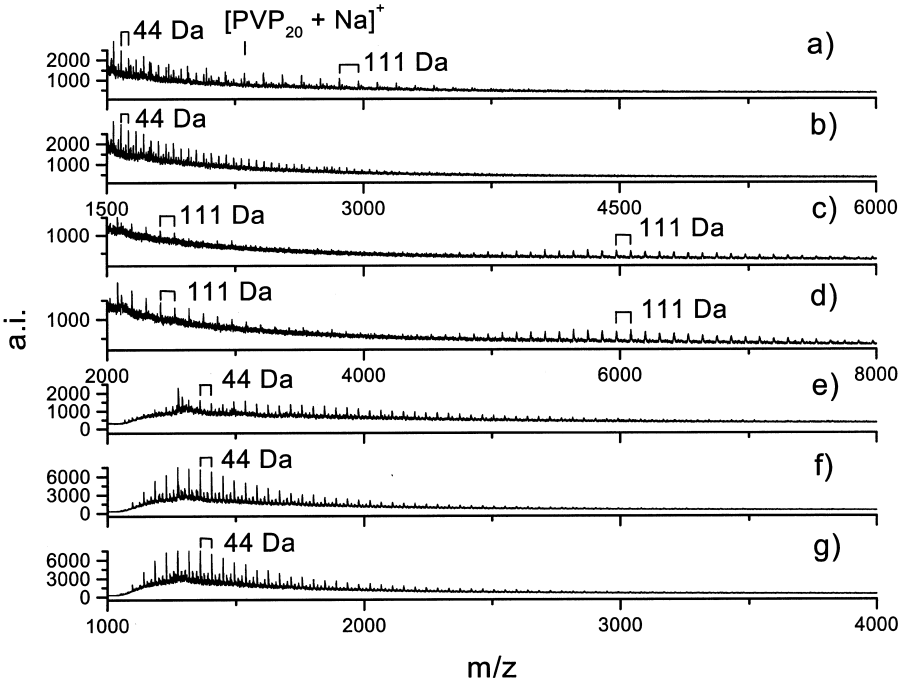

Fig. 8. MALDI-TOF mass spectra of the adsorption experiment of the GPC-fraction of PVP K17 (5–8 kDa (+Na)): (i) PVP-fraction spiked to sludge–Milli-Q (a) 1 h, (b) 2.5 h; (ii) control experiment PVP-fraction spiked to Milli-Q (c) 2.5 h, (d) 32 h; (iii) control experiment sludge–Milli-Q (e) 1 h, (f) 2.5 h, (g) 32 h.

served with a repeating unit of 44 Da which could be attributed to polyethylene glycol (PEG). The verification was easily obtained by the control experiment where the sludge sample was shaken with water in the absence of PVP (Fig. 8d: 1 h). Hence, there is a visible increase observed in the signal intensity of PEG between 1 h (Fig. 8e) and 2.5 h (Fig. 8f) which is then constant during the course of sampling (Fig. 8g: 32 h). The high constancy is recognizable through identical signal intensities in the observed MALDI-TOF mass spectra applying the anchor target device and emphasizes on the high reproducibility of this device. This control experiment showed that the additional polymer distribution indeed results from PEG that was initially present in the applied sewage sludge and dissolved during the adsorption experiment of PVP. PEG is known to be formed as a metabolite of the biodegradation of polyethoxylated surfactants such as alcohol ethoxylates [29].

4. Conclusion

The outstanding advantages of MALDI-TOF-MS are found in the robustness for unequivocal determination of PVP spiked to an environmental matrix such as river water. The use of this method also allows a less time-consuming enrichment procedure. The disadvantage of MALDI-TOF-MS is in particular the non linearity between laser power and concentration for quantitative PVP investigations. These poor MALDI-TOF performances as well as the sensitivity were improved applying the anchor target device for MALDI-TOF-MS and suggests to be valuable for future degradation studies. The limitations that still remain, are the high polydispersities of the different PVP samples (e.g. $D \sim 10$ in pharmaceutical applications, $D \sim 15$ in laundry detergents) which makes MALDI-TOF-MS only applicable after a successful GPC fractionation.

The biodegradation of PVP can be completely excluded with these FBBR degradation experiments monitored with MALDI-TOF-MS whereas the adsorption experiment showed that PVP rather complexes with the sludge. Nevertheless, it cannot be ruled out that this poor biodegradability results from the end group of the investigated PVP sample and that with a different end group a higher degradation may be obtained. These investigations showed that MALDI-TOF-MS can be used as an extended detection method for FBBR experiments and thereby enlarges the analytical window regarding polymeric analytes and thereby makes them accessible for environmental fate studies. The results of these investigations to the biodegradability and the adsorption behavior of PVP allow to assume that this polymer is eliminated from the dissolved phase in wastewater treatment plants by adsorption onto sludge due to its extraordinary complexation property. Extrapolating these results, the question of the final fate and destiny of the adsorbed PVP still remains to be answered.

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