

A First Pilot Study on the Sorption of Environmental Pollutants on Various Microplastic Materials

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Abstract

With the drastic increase in plastic production, the input of plastic particles into the environment has become a recognised problem. Xenobiotics are able to sorb to polymer materials, and this process is further enhanced where they encounter microplastics (plastic fragments <5 mm). In this work we studied the sorption of metformin, a type-2 diabetes drug, and difenoconazole, a fungicide, onto the virgin polymer materials polyamide (PA), polypropylene (PP), and polystyrene (PS). Additionally, PP was cryo-milled and PA was treated with acid to investigate the influence of an increase in surface area and chemical modification. The material properties were also studied by dynamic scanning calorimetry (DSC), gel permeation chromatography (GPC) and Fourier transform infrared spectroscopy (FTIR). Sorption experiments were performed on the basis of a full factorial design examining the effect of agitation, pH value, and salinity. Experimental results showed that difenoconazole sorbs readily to all microplastics, whereas the more polar analyte metformin did not show any affinity to the materials used. For difenoconazole the governing factor in all cases is agitation, while both pH and salinity exhibited only a slight influence. The modification of polymers leads to enhanced sorption, indicating that an increase in surface area (cryo-milled PP) or inner volume (acid-treated PA) strongly favours adsorption. Moreover, long-term experiments demonstrated that the time until equilibrium is reached depends strongly on the particle size.

Keywords: Difenoconazole; Metformin; Plastic debris; Polymer; Dynamic scanning calorimetry

Introduction

Over the last decades, plastic has become one of the most important materials in a wide variety of fields. Global plastic production has risen sharply from 1.7 million tons in 1950 to 311 million in 2014 [1,2]. The polymers with the highest production volumes are polyethylene (PE), polypropylene (PP), polystyrene (PS), poly(ethylene terephthalate) (PET) and poly(vinyl chloride) (PVC) [3].

Due to the light weight and the durability of plastics, they are used extensively for packaging and in the construction industry [2]. Through these applications the uncontrolled input of plastic litter (macroplastic, >5 mm) into the environment has increased distinctly [4]. There the plastics decompose slowly due to aging processes, often triggered by (photo-)oxidation and mechanical abrasion [5]. Thus small polymer particles, also called secondary microplastics (<5 mm), are formed. Primary microplastics, in contrast, are released directly into the environment as ingredients in cleaning products, paints and cosmetics. Although most of the content is removed in waste water plants, microplastics are still present in sewage treatment plants [6-8].

Hydrophobic chemicals like polychlorinated biphenyls [9] and polycyclic aromatic hydrocarbons [10] are known to adsorb on microplastic surfaces [11]. The surface properties of plastics, such as point zero charge, surface area, surface topography, functional groups, and acid-base character, strongly influence the adsorption of other compounds [12]. Wu et al. reported on the adsorption effects of carbamazepine, 4-methylbenzylidene camphor, triclosan, and 17 α -ethinyl estradiol to PE, and evaluated the effects of different salt concentrations and the addition of dissolved organic matter [13]. They determined linear adsorption relationships for all substances, which were attributed to their hydrophobicity. Wang et al. investigated the adsorption of perfluorooctanesulfonate (PFOS) and perfluorooctanesulfonamide (PFOSA) on PE, PS, and PVC. They showed that the sorption efficiency depends strongly on the molecular structure and the composition of the microplastics [14].

However, the adsorption ability of many important environmental pollutants has not been studied sufficiently. Especially polar analytes, which are found frequently in surface water and in municipal

wastewater, have not been considered in publications. In this study the polar antidiabetic drug metformin and the non-polar triazole fungicide difenoconazole were selected as model compounds for interaction studies with microplastics. Relevant data on the two substances are shown in Table 1. Metformin is the drug of choice for treating type-2 diabetes. More than half of the total amount of pharmaceuticals in the environment consist of the antidiabetic agent metformin and its major transformation product guanylurea [15]. In the year 2013, 598 million standard daily doses of metformin were prescribed in Germany [16]. First indications are that metformin could potentially act as an endocrine disruptor in male fish under chronic exposure [17]. Difenoconazole belongs to the group of triazole fungicides that are widely used in agriculture. In 2014, their market share in Germany was 19 %, making them the most commonly used organic fungicides [18]. Difenoconazole is generally perceived as persistent [19]. Furthermore, studies by Mu et al. suggest that difenoconazole acts as an endocrine disruptor [20-22].

Due to their high occurrence and suspected endocrine activity, it is important to investigate the adsorption capacities of these compounds on microplastics in order to evaluate possible transportation routes or accumulation in the food chain.

Only a few publications systematically investigate the influence of different surface water conditions like pH value, salinity, and agitation and their influence on the adsorption capacity on microplastics [14,23].

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Received March 23, 2017; **Accepted** March 28, 2017; **Published** March 31, 2017

Citation: Goedecke C, Mülów-Stollin U, Hering S, Richter J, Piechotta C, et al. (2017) A First Pilot Study on the Sorption of Environmental Pollutants on Various Microplastic Materials. J Environ Anal Chem 4: 191. doi:10.4172/2380-2391.1000191

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In this study the adsorption of metformin and difenoconazole on polypropylene (PP), polystyrene (PS), and polyamide (PA) microplastics was studied. These three materials were chosen because of their relevance to the environment and the differences in their material properties. PS and PP are often used as packing materials. The densities of the materials vary around that of water. PS and PP are hydrophobic polymers with glass transition temperatures T_{glass} below and above those in the experimental design (Table 2). PS is an amorphous polymer, whereas PP is semi-crystalline. PA is also known as nylon, and exhibits excellent mechanical properties, especially as fibres. In this study PA6 was used, as it has a more hydrophilic character. Due to the presence of polar functional groups in PA, an affinity to metformin was expected. The experiments were performed on virgin and aged pellets. Milled PP and acid-treated PA, both of which were characterised physico-chemically, were used to simulate aged microplastics. The parameters salinity, pH, and agitation were varied on the basis of realistic natural conditions and the effects on the sorption of the substances were evaluated.

Experimental

Chemicals and standards

Metformin hydrochloride (97 %) and formic acid (reagent grade, ≥ 95 %) were purchased from Sigma Aldrich (Taufkirchen, Germany). *N*-butylbiguanide hydrochloride at a purity of 95 % was received from Select Lab Chemicals GmbH (Bönen, Germany). Methanol

and acetonitrile of HPLC grade were obtained from OMNILAB Laborzentrum GmbH & Co. KG (Bremen, Germany). Difenoconazole (98.5 %) and deuterium-labelled difenoconazole ($100 \mu\text{g mL}^{-1}$ in acetonitrile) were acquired from Dr. Ehrenstorfer GmbH (Augsburg, Germany). Stock solutions of metformin hydrochloride and the internal standard buformin (*n*-butylbiguanide hydrochloride) were prepared by dissolving an appropriate amount of the substances in ultrapure water to reach a concentration of about 10 mg mL^{-1} . The working solutions were made by diluting the stock solution to achieve a final concentration of $100 \mu\text{g mL}^{-1}$. The stock solution of difenoconazole was prepared by dissolving 10 mg of the original substance in 10 mL of methanol. The solution was diluted to reach a final concentration of $0.02 \mu\text{g mL}^{-1}$ for the spiking solution. $100 \mu\text{L}$ of the spiking solution were added to 50 mL of ultrapure water to yield the working solution. The internal standard solution of deuterium-labelled difenoconazole was made by diluting the purchased solution with methanol to yield a concentration of $2.5 \mu\text{g mL}^{-1}$.

Polymer materials

The polymer materials for sorption experiments were used as granulate (3-5 mm). All materials, i.e., a polystyrene (PS), a polypropylene (PP) as well as a polyamide 6 (PA6), were kindly provided by partners of PlasticsEurope (Frankfurt, Germany) or originated from previous projects at BAM. None of them contained additives for specific applications (i.e., long-term antioxidants, flame retardants, reinforcement additives, pigments, etc.). PP and PS are

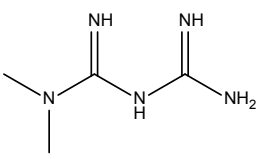
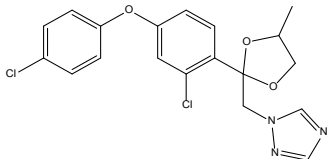
	Metformin [22]	Difenoconazole [19]
		
pKa	10.23, 12.33	1.07
logP	-4.30	4.36
water solubility	10^6 mg L^{-1}	15 mg L^{-1}

Table 1: Structures and properties of the model substances.

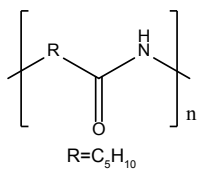
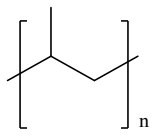
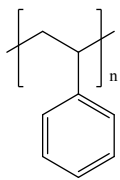
Polymer	Structure [24]	Glass transition temperature/ $^{\circ}\text{C}$ [24]	Density/(g cm^{-3})
PA6		~78	1.02-1.16
PP		0-20	0.83-0.92
PS		90-110	1.04-1.10

Table 2: Structures and glass transition temperatures of several polymers.

hydrophobic polyolefin materials with densities of $\sim 0.90 \text{ g cm}^{-3}$ and $\sim 1.05 \text{ g cm}^{-3}$, respectively, whereas PP displays a semi-crystalline phase morphology. PS is completely amorphous, with a $T_{\text{glass}}=95^\circ\text{C}$. The properties of PA6 (density of $\sim 1.14 \text{ g cm}^{-3}$) are dominated by the polar amide bonds in the chain. PA is semi-crystalline and has a $T_{\text{glass}}=40\text{--}60^\circ\text{C}$, whereas in PA6 T_{glass} depends strongly on the water content in the sample (Table 2). For a compilation of the material properties see Table 2.

Milled PP: Virgin PP pellets were milled at 16000 rpm in a cryogenic mill (Retsch, ZM 200, Haan, Germany) under nitrogen cooling, using a 2 mm ring sieve. Sieving analysis found that around 55 % of the particles have dimensions of 2.0 to 0.6 mm, ~ 40 % are between 0.2 to 0.6 mm and only 5 % are smaller than 0.2 mm.

Acid-treated PA: To simulate aged microplastics, virgin PA granulates were submerged for 24 h in a mixture of HCl ($w=12$ %) and 20 % acetone [24,25]. For most polymers the aging process is characterised by a reduction in molecular weight. This degradation process is often initiated by oxidative attack. However, this process is time-consuming, because polymers are usually equipped with antioxidants that suppress this reaction mechanism. For our lab experiments we avoided this procedure by using treatment with an HCl/acetone solution. After filtering, the treated pellets were neutralised with sodium hydroxide solution and rinsed with water several times. Finally, the microplastics were dried overnight at 80°C . Afterwards the previously compact granulate pieces had changed into brittle flakes. The aging process was characterised using FTIR, DSC, and GPC analysis.

Sample preparation and analysis

One gram of the respective microplastic pellets was placed in a glass vial and 50 mL of the appropriate standard solution was added. The pH values of the samples were adjusted with sodium hydroxide solution (0.1 M) and hydrochloric acid (0.1 M). The salinity was regulated by the addition of NaCl to reach final salt concentrations of 0.1 wt % and 3.5 wt %. The samples were stored or agitated on a horizontal shaker for a defined time period.

All experiments were carried out in duplicate. Additionally, one sample without microplastics was treated in the same way, in order to determine the adsorption of the analytes onto the glass surface. This value was then subtracted from the adsorption values for polymer materials.

The analysis of metformin was performed using high-performance liquid chromatography with diode array detection (HPLC-DAD). The microplastics were separated by decantation. Afterwards, 200 μL of the samples and 200 μL of bufornin standard solution were combined in a sampling vial and 600 μL of ultrapure water added.

For gas chromatography mass spectrometry (GC-MS) measurements of difenoconazole, 100 μL of deuterium-labelled difenoconazole internal standard solution were added to the sample and the solution was filtered using a folded filter (MN 615, 0.16 mm, 70 g m^{-2} , Macherey-Nagel GmbH & Co. KG, Düren, Germany) to remove the microplastics. The filtrate was extracted for 15 minutes using 10 mL of ethyl acetate. Subsequently, 10 mL of cyclohexane were added and the filtrate was extracted again for 15 minutes. The organic layer was separated and dried over sodium sulfate. The solution was then concentrated to 1 mL under a gentle stream of nitrogen prior to GC-MS measurement.

Analytical methods

Dynamic scanning calorimetry: The dynamic scanning calorimetry

(DSC) measurements were carried out with a DSC 7020 (Seiko, THASS, Germany) equipped with an autosampler. About 3 mg of the sample were weighed in an aluminium pan and heated up from -80 to 260°C . Subsequently, the sample was cooled to -80°C and reheated to 260°C . For all measurements a heating rate of $10^\circ\text{C min}^{-1}$ was used under a nitrogen atmosphere (50 mL min^{-1}). In DSC measurements the first heating curve reflects the “thermal history” of the material (in this case, also the exposure to an aqueous solution), whereas the first cooling and second heating can be used for material characterisation.

Gel permeation chromatography: The gel permeation chromatography (GPC) measurements were performed with a Merck-Hitachi L 7100 HPLC-pump and hexafluoroisopropanol (HFIP) as eluent, equipped with a refractive index detector (ERC-7510). The three columns (1 x HFIP gel $30 \times 0.8 \text{ cm}$ and 2 x PL-HFIP gel $25 \times 0.46 \text{ cm}$) were heated to 35°C using a Merck-Hitachi L-5025 column thermostat. Samples dissolved in HFIP with a concentration of 5 mg mL^{-1} were prepared and filtered through a $2 \mu\text{m}$ PTFE filter. 100 μL of the solution were injected into the system. The flow rate was 0.5 mL min^{-1} . The molar mass distribution (M_n , M_w) was calculated using WinGPC software (PSS GmbH, Mainz). Poly(methyl methacrylates) (PMMA) samples of different molar masses were used as calibration standards.

LC-DAD: Analysis of metformin was carried out using an Agilent Technologies HP-1100 Series (Agilent Technologies, Waldbronn, Germany) HPLC system. The HPLC was equipped with a DAD operating at a detection wavelength of 232 nm. Chromatographic separation was achieved with an Acclaim Polar Advantage C16 analytical column ($4.1 \times 250 \text{ mm}$, $5 \mu\text{m}$ particle size, Dionex) at a flow rate of 1 mL min^{-1} . The mobile phases consisted of A: H_2O with 10 mmol $\text{CH}_3\text{COONH}_4$ and B: MeOH/ACN, 1/1 (v/v). The gradient was 10 % A at 0 min, 90 % A at 6 min, 90 % A at 10 min, 10 % A at 15 min and 10 % A at 18 min.

GC-MS: For analysis of difenoconazole, an Agilent GC 7890A coupled to a 5975C inert XL MSD (Agilent Technologies, Waldbronn, Germany) was used in combination with an MPS2XL Multipurpose Sampler (Gerstel, Mühlheim an der Ruhr, Germany). He 5.0 with a flow rate of 1 mL min^{-1} was used as the carrier gas, and the column was a DB5-MS ($30 \text{ m} \times 250 \mu\text{m} \times 0.25 \mu\text{m}$, Agilent Technologies). Measurements were done in selected ion mode with an EI source set to 70 eV. The MS ion source was maintained at 230°C . One μL of the sample was injected using a KAS4 injector (Gerstel GmbH & Co. KG, Mühlheim an der Ruhr, Germany) which was programmed to start injection at 40°C and ramp up to 275°C at a rate of 12 K s^{-1} , maintaining the target temperature for 5 min. The temperature of the column oven was programmed as follows: 60°C to 210°C at 20 K min^{-1} , hold for 5 min, at 10 K min^{-1} to 255°C , hold for 5 min, at 4 K min^{-1} to 310°C , hold for 10 min. The total runtime was 45.75 min. The signals for the native and the deuterated diastereomers were each integrated separately. For the adsorption values both signals were considered in total.

The sorption of the pollutants was determined via the remaining content of the analytes in the aqueous phase. Therefore, the difference between the ratio of analyte to internal standard at the beginning of the experiment as opposed to the ratio after the end describes the adsorption on the microplastics. The blank value (adsorption on glassware, see section 2.3) was subtracted from this value.

Experimental design

A full factorial design with two levels for the three design variables “agitation”, “pH”, and “salinity” was created for all microplastics (PA, PP, PS, PA-acid, and PP-milled) using the Design Expert 9 (Stat-Ease).

All designs were tested with true replicates but did not contain centre samples. The designs were evaluated by analysis of variance (ANOVA), including second and third order interactions using “adsorption” as the response variable.

Results and Discussion

Selection of experimental parameters

Apart from the polymer materials used for the studies, pH value, salinity, and agitation were identified as the main parameters influencing the sorption behaviour of analytes. As the pH value of surface waters usually varies between pH=6 and pH=8 [26], these values were chosen as the lower and upper limits. The salt contents were selected to represent freshwater (0.1 %) and sea water (Mediterranean Sea: 3.5 %) [26-28]. Not all of the salt content in bodies of water is composed exclusively of sodium chloride. Nevertheless, NaCl is by far the most abundant component and has previously been used without further additions to model salt waters [29]. Furthermore, standing bodies of water (agitation 0 min⁻¹) were compared with turbulent waters (240 min⁻¹) [30].

Adsorption onto virgin polymers

Since difenoconazole is a predominantly hydrophobic analyte (Table 1), a pronounced sorption affinity to all selected polymer materials was expected. Among the polymers tested, PS was of special interest because of possible interactions of the π -electron systems [31,32] and its amorphous structure [24].

Figure 1 illustrates the results of the 24-hour adsorption experiments using virgin polymer material and difenoconazole solution. The graphs depict the adsorption of the analyte on the microplastics as a function of pH, salinity, and agitation. The values for pH and salinity are denoted on the right-hand axis. The absence and presence of agitation are designated by open or filled columns. At first glance it is noticeable that for PA and PP the filled columns correspond to higher adsorption values in all cases. The values obtained for PS exhibit higher variations in the adsorption intensities than PP and PA.

In order to provide a sound hypothesis about the sorption process of difenoconazole on microplastics, the data were evaluated by ANOVA, which yielded significant models in all cases. The results are provided in Table 3, which summarises both non-significant and significant model terms; the latter are marked with asterisks. From Table 3 it can be seen that among the tested design variables, “agitation” has the

strongest effect on adsorption for all microplastics, followed by salinity and pH, both with much weaker effects. The parameter “pH” was a significant term only for PS, but not for PP and PA. In addition, some significant second-order interactions with low effects were identified, but no significant third-order interaction was found. In general, these ANOVA results are in line with the graphs.

Especially for PA and PP, higher adsorption can be observed for the agitated samples than for the non-agitated samples. With these polymers a maximum adsorption of up to 25 % is reached when samples are agitated. Figure 1 shows a trend of increased adsorption at higher salinity for PA and PP. However, the effect of agitating seems to be inverse to the effect of varying pH. When samples are agitated and salinity is high, a change in pH does not lead to a change in adsorption on PA. If the samples are not agitated, increasing the pH value results in higher adsorption. Nevertheless, according to ANOVA the pH value is not a significant term for PA and PP.

For PS in general, the adsorption levels reached in the non-agitated samples are higher than for PA and PP. Although upon agitation an increase in adsorption can be observed, the relative increase is less than for the other polymers. Moreover, the effect of salinity on PS is now inverse the effect on PA and PP (negative sign for term “salinity” in Table 3).

We suppose that the effect of agitation may be related to the inherent properties of the polymer materials, for example their floating behaviour: PA sank to the bottom of the vial, PP floated, and PS was located both on the surface of the water and the bottom of the vial (Table 2).

Adsorption of difenoconazole in non-agitated solutions was much

	PP	PA	PS	PPmilled	PAacid
A: agitation	+4.11*	+6.28*	+2.94*	+33.04*	+24.76*
B: pH	+1.71	-0.37	-1.20*	-3.30*	
C: salinity	+1.90*	+2.74*	-2.10*	+2.35*	1.12
AB		+2.29*			
AC		+2.97*	-2.33*	1.19	+2.24*
BC	+1.85*	+0.47	+1.31*	-2.05*	
ABC					

Table 3: Parameters of equation in terms of coded factors (+1, -1) derived from ANOVA. Significant variables, i.e., when p-values are found lower than 0.05, are marked with an asterisk.

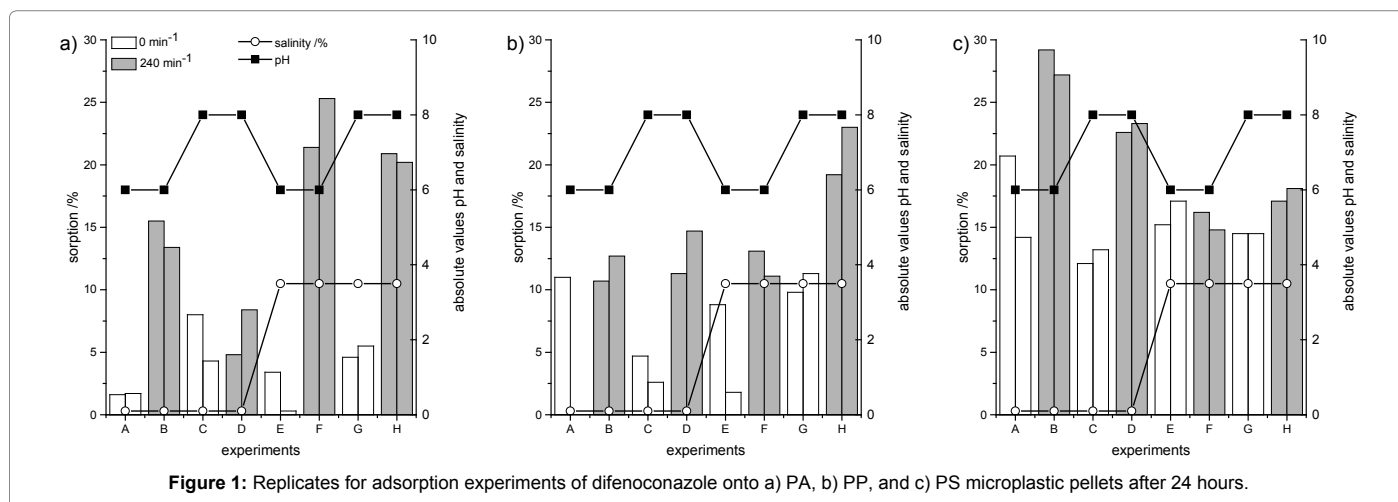


Figure 1: Replicates for adsorption experiments of difenoconazole onto a) PA, b) PP, and c) PS microplastic pellets after 24 hours.

higher for PS than for PP and PA. PS is the only polymer tested that is completely amorphous (Table 2). Therefore, adsorption occurs not only on the surface, but may also result from effects like permeation and diffusion into the free volume of the polymer. The observed influence of salinity on the adsorption could be attributed to either a salting-in or salting-out effect of difenoconazole. It can also be assumed that the salt content of the matrix, in interplay with the structure of the respective polymer, is responsible for the changes in adsorption.

Difenoconazole is a weak base (Table 1). Still, under the parameters used in this study it is mostly neutral, thereby diminishing the effect of a change in pH on adsorption. As the acetate moiety of difenoconazole is also stable under these conditions, no changes in the interaction of the model compound and the polymers are expected due to a chemical change in the molecule.

There are already several reports on the interaction of the employed polymer materials with non-polar analytes. In the case of PP, a high sorption capacity to PAH has already been described. According to Krüger et al., PP containers may reduce PAH recovery by up to 80 % [33]. In a similar study, it was found that higher salinity favours the sorption of phenanthrene ($\log P=4.46$) [34] onto PP [35]. Rochman et al. showed that more low-molecular PAHs ($\log P < 5$) sorbed to microplastics made from PS than from PP [10], supporting the results of this study.

With regard to pH, fewer studies with definitive results have been published. Fotopoulou et al. showed through mass potentiometry that PP does not exhibit any acid-base interactions [12]. Interaction between PP and difenoconazole should therefore be more or less the same at pH=6 and pH=8. PA is influenced by pH, however, and may undergo acid-base reactions or even form complexes with metal cations [36]. Still, under the experimental conditions used in this study, no strongly enhanced interaction with difenoconazole under changed pH is to be expected, as proven by our experiments. In their studies dealing with the adsorption effects of PFOS and PFOA, Wang et al. suggested that salinity may play an important role in sorption processes because it can enhance or diminish repulsive electrostatic forces.

Concerning the hydrophilic analyte metformin, under the given parameters no significant adsorption to PA could be observed after 24 hours (data not shown here). Below pH=9, the polar analyte metformin ($\log P: -4.30$, $pK_a: 10.23$ and 12.33 , see Table 1) carries a double positive charge [15]. This leads to increased solubility in water and inhibits interaction with non-polar microplastics. Even though the chemical structure of the polymers may also be influenced to some extent by changes in pH, the combined effects do not seem to lead to increased metformin sorption.

It was expected that salinity might strongly affect the adsorption behaviour of metformin because of its charges. However, no change in adsorption could be observed at 3.5 % salt content, which is an environmentally relevant value. Experiments with this salinity were also repeated at pH=10 and pH=3, but did not lead to any detectable adsorption, either. To remain close to environmental conditions, experiments with more extreme pH values were not carried out. In order to avoid the possibility that degradation was not observed due to excessively high concentrations, experiments were carried out with more dilute metformin solutions ($10 \mu\text{g mL}^{-1}$ and $1 \mu\text{g mL}^{-1}$).

Influence of particle size

In order to simulate the adsorption on microplastics with an environmentally relevant size distribution, experiments were repeated using milled PP. The results of the adsorption studies using

difenoconazole are shown in Figure 2. Agitation of samples leads to a strong increase in adsorption compared to the non-agitated samples. It can be assumed that increasing the surface area has the strongest influence on adsorption. In addition, pH, salinity, and the combined effect of both (BC) have a significant effect on the adsorption of difenoconazole (Table 3).

Compared to the virgin PP pellets, adsorption on the milled material increased by a factor of four, to approximately 80 %. Previous studies indicated that sorption on PP is governed by a surface mechanism [35]. Milling of the microplastics leads to a strong increase in the surface area (according to the rough estimation of particle dimensions, by a factor of about 5 to 14). Thus, the sorption processes on the surface of the particles are intensified. In addition, diffusion pathways into the interior of the PP pellets could become accessible due to the milling process, just as Karapanagiotti et al. proposed for eroded PP [37]. Our results are consistent with the studies by Bakir et al., who suggested that the sorption equilibrium of organic pollutants on plastics is reached much faster for smaller particles [38].

In order to assess whether the polar analyte metformin had not shown adsorption to any of the virgin microplastics in the previous experiments due to a limited surface area of the polymers, experiments were repeated with milled PP. Since no adsorption of metformin was observed after 24 h, the experiments on milled PP were performed over a period of 34 days with a pH of 8 and a salt content of 3.5 % (for the general practical implementation, see section 2.3). However, no significant adsorption was observed.

Influence of polymer treatment

Figure 3 shows the results of the sorption experiments on difenoconazole with the chemically treated PA. In all cases, agitated samples exhibited adsorption about seven times higher than did the non-agitated samples. This implies that agitation has the most important influence on the adsorption effect and is further in line with the results of ANOVA, suggesting that, besides agitation, only the interaction of agitation and salinity has a significant effect (Table 3). The adsorption of difenoconazole onto the treated PA is about three times higher than that of the virgin pellets.

The FTIR spectra (see supporting material) do not show any difference between the two materials, indicating that no significant

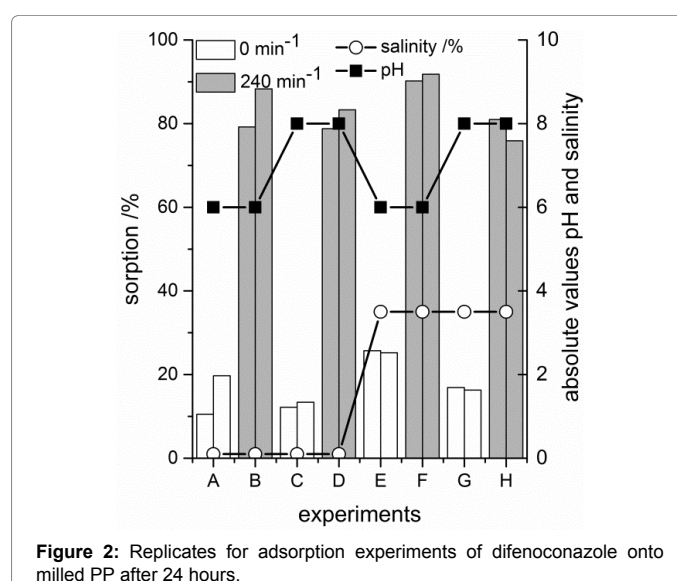


Figure 2: Replicates for adsorption experiments of difenoconazole onto milled PP after 24 hours.

change in the chemical composition (oxidation) of the material took place. In contrast, DSC and GPC analysis show clear differences (Figure 4). The comparison of the GPC measurements of the virgin and the aged PA6 reveals a clear shift of the peak towards lower molar masses ($M_n=42.700 \text{ g mol}^{-1}$ to $30.700 \text{ g mol}^{-1}$, $M_w=84.600 \text{ g mol}^{-1}$ to $66.400 \text{ g mol}^{-1}$). The reduction of all relevant key parameters by about 25 % indicates the systematic chain scission of all polymer molecules.

The result of GPC investigation – the reduction in molar mass – is illustrated in DSC measurements by the shift of the crystallisation peak to higher temperatures during the cooling process, as well as by the shift of the melting peak and the glass transition temperature to lower temperatures during the second heating process [39] (data not shown). However, the first heating cycle offers new results. A small endothermic peak for the virgin PA 6 and a broad peak for the aged PA6 can be observed below 100°C . These peaks are caused by the vaporisation of water. With a sample weight of 2.489 mg and an evaporation enthalpy of water of 2.257 J g^{-1} , a water content of about 1.5 % can be calculated. Around 220°C the endothermic signal of melting is observed. The melting process is related to the crystalline phase of the material. For

the aged PA 6 the melting enthalpy and therefore the crystallinity is decreased by 39 % compared to the virgin PA 6.

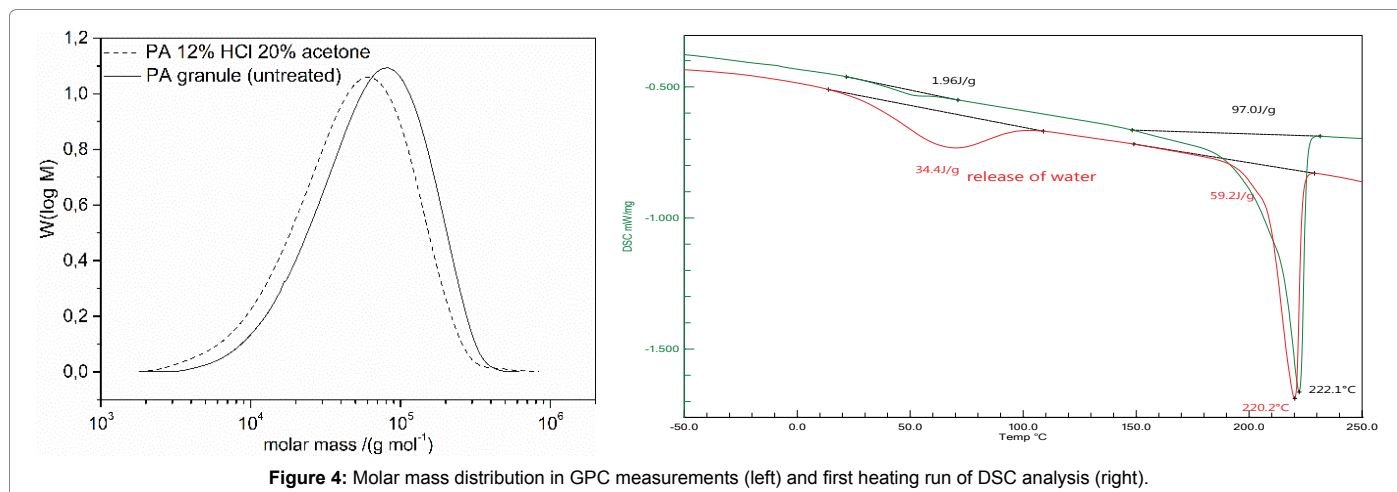
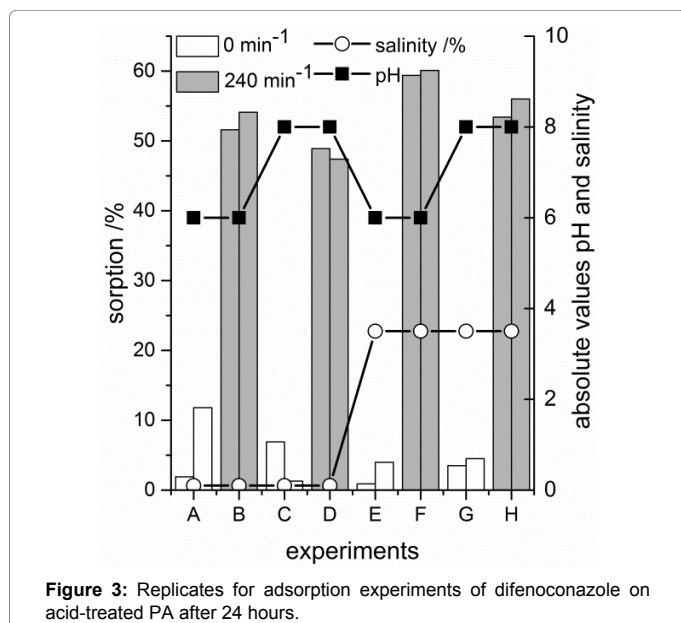
It can be concluded that treatment with the HCl/acetone solution degrades the molecular weight, but also results in increased water uptake and reduction of crystalline content in the sample. In first approximation, this can be interpreted as a swelling of the molecular chain arrangement. The increased adsorption of difenoconazole onto the treated PA must be related to the reduction of the molecular mass and crystallinity, due to the cleavage of the polymer chains from an otherwise non-altered chemical structure. We therefore suppose that the treatment led to an increase in the inner volume of the polymer particles.

Long-term experiments

Adsorption processes in the environment have a lot of time to unfold. After a certain period, a dynamic equilibrium can be reached, in which desorption and adsorption take place at the same time with the same velocity. To provide a statement about the time until the equilibrium was reached for difenoconazole in interaction with the microplastics used, samples were evaluated over the course of 28 days. In Figure 5 the adsorption of difenoconazole on several microplastics is shown as a function of time. When an adsorption curve reaches a plateau, equilibrium has been achieved during the sampling period.

For the virgin pellets, similar equilibrium rates can be observed for PA and PS (Figure 5a). This indicates that the physico-chemical properties of these different polymers (amorphous morphology of PS, chemical structure of PA) are of minor importance, or that the different properties compensate for each other. PP adsorbs difenoconazole more slowly.

In contrast to this, the influence of the particle size is obvious for PP: With the milled PP, 91 % of difenoconazole had sorbed to the microplastics after 24 hours (Figure 5b). Further fluctuations in concentration can be attributed to measurement uncertainties; therefore the equilibrium is approximated. Similarly, for acid-treated PA the equilibrium is already in place after seven days, whereas it is not reached until the 18-day mark by untreated PA. This surprisingly fast adsorption of PP powder and treated PA indicate a complex mechanism of adsorption and desorption of the permeating species into the polymer particles, as well as changed diffusion, especially through the treated polymer. Because no particle size or particle form



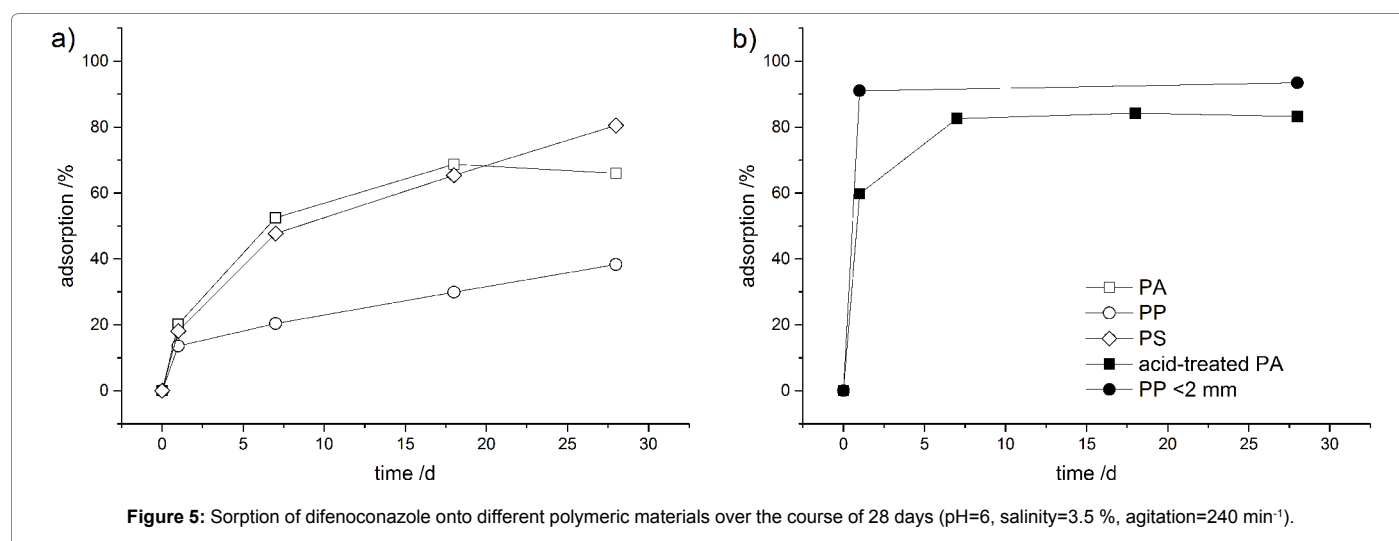


Figure 5: Sorption of difenoconazole onto different polymeric materials over the course of 28 days (pH=6, salinity=3.5 %, agitation=240 min⁻¹).

distribution is documented for these materials, no further conclusions can be drawn; however, this result indicates the urgent need for exact particle descriptions when microplastic powders are used.

For their studies on the adsorption of different organic compounds on microplastics, Hüffer et al. determined the equilibrium of the sorption process using naphthalene ($\log P=3.29$) [23]. They found that it took longer to reach equilibrium on PS than on PA (17 d vs. 5 d). This verifies the results of the presented study. As for adsorption of phenanthrene ($\log P=4.53$) on PP pellets, it has been reported that it took 20-40 days to reach equilibrium [35]. It is unclear whether a similar value is obtained for difenoconazole, as the duration of the experiment was not sufficiently long.

Conclusions

For the first time the adsorption behaviour of difenoconazole and metformin on microplastics was evaluated systematically. The parameters agitation, salinity, and pH were investigated concerning their influence on the adsorption of metformin and difenoconazole on microplastics. Experiments were based on a 2³ full factorial design with the above parameters adjusted to environmentally relevant values. PA, PP, and PS were used as virgin pellets. In addition, PP was cryo-milled to increase its surface area and PA was chemically pre-treated to simulate a loss of molecular weight.

Difenoconazole shows sorption onto all hydrophobic polymers used in the study. The adsorption reached its maximum values when particle size (PP) or molecular weights were reduced (PA). Among the untreated polymers, the adsorption of difenoconazole was highest for PS, followed by PP and PA. This is in line with previous studies on the adsorption of PAHs to different synthetic polymers. Our experimental results showed that adsorption onto all microplastics studied here is strongly governed by agitation. The effect of salinity is comparably small, and pH seems to be of least relevance.

Long-term experiments showed an increase in the adsorption of difenoconazole over the entire experimental period of 30 days. Among the untreated microplastics, PS and PA reached adsorption levels of about 80 % and 60 %, respectively, whereas adsorption to PP amounted to 30 % at the end of the long-term study. As no saturation was reached, further adsorption over longer periods must be assumed.

No adsorption of metformin onto untreated polymer pellets was found under the experimental conditions. It can be assumed that because of the high polarity of metformin, sorption is not energetically favoured. Hence, the accumulation on polymer particles and thus long-range transport in water is not of concern for this analyte.

Acknowledgements

The authors thank J. Falkenhagen and E. Dümichen for GPC and DSC investigations, as well as A. Müller for providing the milled polypropylene.

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