Assessment of ATR-FTIR spectroscopy with multivariate analysis to investigate the binding mechanisms of Ag and TiO_2 nanoparticles to Chelex®-100 or Metsorb™ for DGT technique

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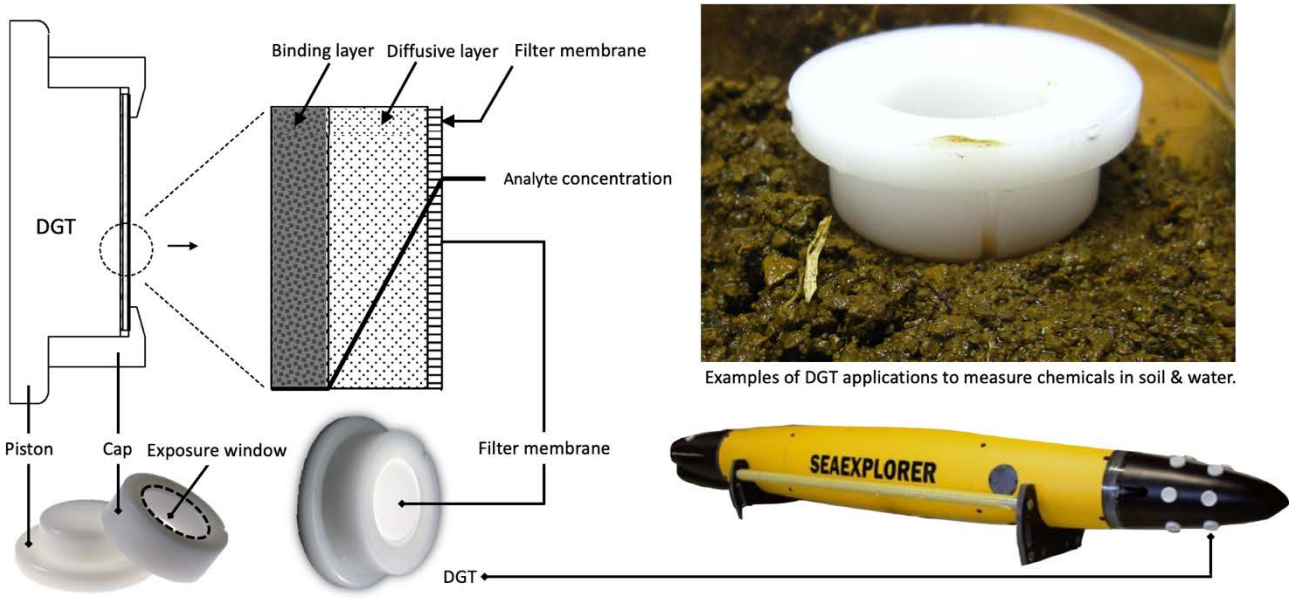
Abstract

Studying nanomaterials' ecotoxicology is not new but there are still gaps in our understanding of their fate in the environment. A major challenge is lack of reliable tools to measure available concentrations of nanoparticles (NPs) in soil and water. Diffusive gradient in thin-films (DGT) is a robust technique for measuring concentration of trace metals in the environment. We have also shown that it could be potentially developed for measuring ZnO NPs[1,2]. To further investigate the suitability of DGT for measuring available concentrations of NPs in soil and water we selected two model nanoparticles, Ag and TiO_2 which are widely used and incorporated in different commercial products. We aimed to understand (1) if two of the DGT binding agents, Chelex®-100 and Metsorb™, could irreversibly retain our model NPs and if yes (2) what might be the differences between bound Ag and TiO_2 NPs and Ag^+ and Ti^{4+} cations. We used ATR-FTIR spectroscopy for this purpose and analysed the IR spectra using principal component analysis and linear discriminant analysis (PCA-LDA), as our pattern recognition tool. The results show that the DGT resins form chemical bonds with silver and titanium nanoparticles and their ionic forms. PCA-LDA demonstrates that the binding mechanisms are statistically different (95% confidence level) among the treatments. The study indicates DGT potential for measuring available concentrations of NPs in the environment and suggests that ATR-FTIR spectroscopy combined with computational analysis could potentially differentiate between chemical species that are retained simultaneously by the DGT device resin layer.

Keywords: Attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy; Diffusive gradient in thin-films (DGT); Linear discriminant analysis (LDA); Principal component analysis (PCA); Ag NP; TiO_2 NP

1. Introduction

Diffusive gradient in thin-films which is often known as DGT technique is a simple, yet accurate and reliable approach in measuring time-averaged concentrations of different chemicals in soil and water [3–6]. A DGT device is comprised of two key components; a diffusive hydrogel layer and a binding layer, which are protected by an external filter membrane and placed on a plastic base. A plastic cap keeps the filter membrane, the diffusive (hydrogel) and binding layer in place on top of the plastic base (piston) as illustrated in Figure 1. The function of each part of the DGT device and detailed features of them have been described extensively in previous studies [7,8].



The DGT technique is based on the concept that the target chemical species pass through the diffusive layer, they are irreversibly retained by the binding resin. With the

Figure 1. Schematic representation of a DGT device and its components in addition to its application in soil and water as illustrated in other research.[6]

known thicknesses of the diffusion layer, diffusion coefficients of the retained species, the surface area of the exposure window, the length of time and ambient temperature during the DGT deployment, the time averaged concentration of retained chemicals can be determined using the DGT equation, which is based on the Fick's law of diffusion.[8]

A crucial step for measuring the time-averaged concentration of a chemical by DGT is its ability to pass through the diffusive layer and being retained by the binding layer. In our previous research we showed that the DGT technique can potentially measure concentrations of manufactured nanomaterials in the environment[2], an approach which was promisingly repeated in other research using the same principles.[9–13] We used combinations of different membranes and diffusive gels to allow certain sizes of particulate matters to enter the device for measuring different targeted chemical species.[3] The most commonly used diffusive gels in the DGT technique are agarose, open pore polyacrylamide and restricted polyacrylamide gels. Each of them has different pore sizes. Some studies suggest that particles as large as 100 nm can diffuse through open pore hydrogel.[14] For the binding layers, Chelex®-100, Metsorb™ and Fe-Oxide gels are often used in the DGT devices.[1,2,8]

We have previously studied the binding mechanism of ZnO nanoparticles (NPs) and Zn^{2+} to Chelex®-100 and Metsorb™ as two commercially available and widely used binding resins.[1,2,5] In this study, we focus on using the same resins for two industrially important and extensively made nanomaterials[16,17]; Ag and TiO_2 nanoparticles and their ionic forms Ag^{+} and Ti^{4+}. Chelex®-100 is an ion-exchange resin with efficient binding surfaces, which stems from its chemical structure. It includes dicarboxylic acid amine ($COOHCH_2$-NH-$COOHCH_2$) with carboxyl groups, which deprotonate in relatively low pH values (pH≈4).[18]

Less information is available about Metsorb™ chemical structure compared to Chelex®. This commercial material is mainly a titanium oxide-based resin with TiO_2 and

$Ti(OH)_4$ as its main constituents and small quantities of other chemicals including polymers[19]. Both titanium oxide/hydroxide constituents in Metsorb™ have a low point of zero charge (PZC, the pH that overall surface charge is neutral); which make its surface to pose overall negative surface charge at the pH of natural environments. These pH values (PZC) are approximately 4.5 for $Ti(OH)_4$ and 6.0 for TiO_2.[5,20,21]

Both Chelex®-100 and Metsorb™ have been used extensively as binding materials in the DGT devices for measuring targeted chemical species in soil and water[6]. We have also shown that these two resins are capable of retaining ZnO nanoparticles and potentially could be deployed for measuring nanomaterials in the environment.[8,22] The performance of Chelex®-100 and Metsorb™ and their reliable function as binding materials in the DGT devices for ionic metals and nutrients have been studied before, nevertheless limited information is available about potential for nanomaterials.[23,24] Studying the biding mechanisms of chemicals to the aforementioned resins help us to better investigate their suitability for using in the DGT technique to measure time averaged concentrations of the nanomaterials in the environment.

The aim of this research is to investigate how Chelex®-100 and Metsorb™ retain four different chemicals, Ag and TiO_2 nanoparticles and their ionic forms Ag^+ and Ti^{4+}. For this purpose, we used attenuated total reflection-Fourier transform infrared (ATR-FTIR) spectroscopy, followed by principal component analysis (PCA) and linear discriminating analysis (LDA) to further process ATR-FTIR data. ATR-FTIR spectroscopy is widely used for exploring chemical interactions at solid-liquid interfaces for both organic and inorganic samples and as a non-destructive technique.[25–27] It will provide information to elucidate the underlying differences between these nanoparticles and their ionic forms chemical bonds to the DGT resins. Ag and Ti nano-particles are widely used in different industries as engineered nanomaterials. Previous studies indicate that after the waste treatment processes,

they likely re-enter the environment and accumulate in soils as their primary sink. This may lead to bioaccumulation in microorganisms and plants and affect the food chain.[5,28] Measuring these nanomaterials concentrations in soil and water helps us to better understand their behavior in the environment.

Silver nanoparticles are well-known for their antimicrobial properties and used in many industries particularly in personal care products and clothing e.g. in textile to eliminate bacteria and odor. These nanoparticles also have unique physicochemical characteristics (e.g. electrical and thermal conductivity), which makes them attractive for many industries, for example food packaging, cosmetics and biomedical related products, that increase their risk of being released to the environment.[29,30] Titanium dioxide nanoparticles are stable in the environment with desirable photocatalytic properties, they have high refractive index, which make them excellent white powder pigments, whit extensive applications as coating or paint materials. Both of these nanomaterials have shown to have adverse environmental impacts when they are released to the environment, especially in high concentrations, which makes probing their concentrations in the environment essential. [17]

Experimental section

1. Material and Methods

2.1 Chemicals

TiO_2 was obtained from EVONIK (AEROXIDE® TiO_2)[31], and Ag nanoparticles were sourced from Institut Català de Nanotecnologia, Spain. The silver nanoparticles were polyvinylpyrrolidone (PVP 9.73%) with primary particle size expected to be approximately 25nm. Detailed properties of these Ag nanoparticles, provided by the above-mentioned supplier, have been described in other studies.[16] The primary particle size of the TiO_2 NPs

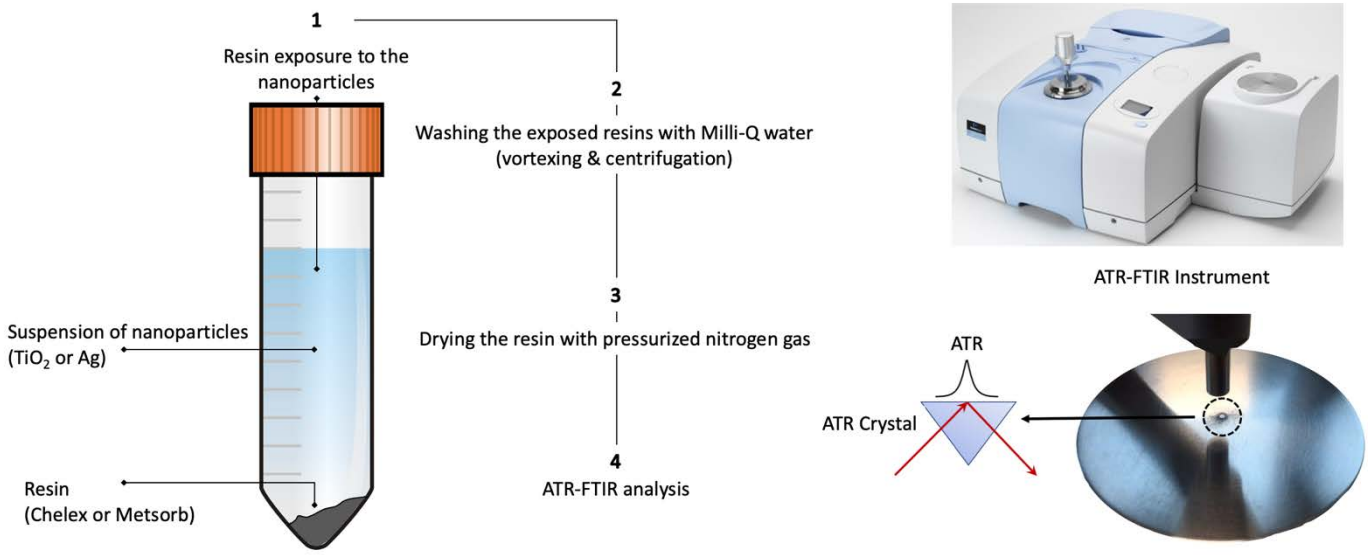
was expected to be approximately 50nm. Point of zero charge (PZC) of Ag is usually below pH≈4, while it is about pH≈6 for TiO_2 NPs,[17,32] which create respectively negative and positive Zeta potential (electrokinetic potential) at the surface of these nanoparticles in this study. Some details can be found in supplementary information (SI). The DGT resin materials, Chelex®-100 and Metsorb™ were purchased from Bio-Rad and Graver Technologies. Before each test, fresh stocks of Ag and TiO_2 nanoparticles dispersions in Milli-Q water were prepared, followed by sonication for at least15 minutes. Fresh stocks of Ag^+ was prepared by dissolving Sigma-Aldrich reagent grade $Ag(NO_3)$ in Milli-Q water. For Ti^{4+} stock solution, acidified Sigma-Aldrich reagent grade $TiCl_4$ was used.

2.2 Sample preparations

Each experimental treatment for ATR-FTIR spectrochemical analysis was prepared in triplicate. For this purpose, 0.5 g of Chelex®-100 or Metsorb™ (binding materials) was exposed to 20.0 ml of 1000.0 µg/l of one of six different treatments; (1) Ag NPs, (2) Ag^+, (3) mixture of Ag NPs and Ag^+ (500 µg/l each), (4) TiO_2 NPs, (5) Ti^{4+}, (6) mixture of TiO_2 NPs and Ti^{4+} (500 µg/l each), which corresponds to six different spectral classes for each of the resins. We used high concentrations for each of these treatments to enable reliable detection by ATR-FTIR spectroscopy. The pH for all the experiments in this study was approximately 5.8. For Ti^{4+} stock solution, which had been prepared from acidified $TiCl_4$, the pH was adjusted by adding very diluted NaOH solution (reagent grade). The pH were monitored for 48 hours throughout the experiments.

Each sample of a fresh chemical solution with the resin was shaken for over 3 hours on a rotary shaker at 150 rpm. Then they were centrifuged for 20 minutes at 3500 rpm and the supernatant was discarded. The resins were washed by adding 50 ml of Milli-Q water, shaking for further 30 minutes and centrifugation as before. This process was repeated three

times to remove residuals that may be attached physically on the resins but not retained by chemical binding. The samples were dried by pressurized nitrogen gas (0.4 bar) and a small portion of each dry sample was put on the ATR crystal for ATR-FTIR spectroscopy analysis as illustrated in Figure 2. The samples prepared for the analysis completely covered the ATR crystal and provided a thickness of over 3 µm to eliminate any interference that could be



caused by the compression rod that keeps the samples in place.

Figure 2. Schematic representation of the experimental procedure. Exposing Chelex®-100 and Metsorb™ to Ag and TiO_2 NPs suspensions and their ionic forms for spectral acquisition

2.3 Spectra acquisition and data processing

A PerkinElmer Spectrum 100 FTIR Spectrometer was used for data acquisitions (Figure 2). Each experiment was conducted in triplicate and for each sample, 30 spectra were acquired at 2 cm^{-1} spectral resolution. The IR spectra covered 600-4000 cm^{-1} range, were divided into three different spectral regions: region one 600-800 cm^{-1}; region two, 800-2200 cm^{-1}; and, region three, 2600-4000 cm^{-1}. To compare the spectral classes, baseline corrections

(rubber band) and vector normalization were used for all of the regions for each treatment. Details of these techniques and available tools to perform these analyses have been described in previous studies.[33–35]

Following this step, the spectral data were further processed using principal component analysis (PCA) coupled with linear discriminating analysis (LDA), which often referred to as PCA-LDA. PCA and LDA both are statistical procedures and data transformation techniques used in a range of engineering concepts to classify different data and identify patterns. PCA can be described as a linear dimensionality reduction method to project the data into the directions of highest variability (where the data is most spread), while LDA computes the highest possible discrimination between different classes of data which helps us to classify the data accurately.[36,37]

In this study we used PCA to reduce the number of variables in spectral data (*i.e.*, absorbance intensities at different wavenumbers) to only few factors that can capture >95% variance of the dataset. If the within-class variation is larger than what is found between classes, LDA can be applied to the output from PCA to minimize the within-class variation and maximize between-class variation. This allows us to identify the most important discriminating information and remove interference from the data as described in previous research.[1,2,33] In other words, this method helps us to identify class differences through the generation of scores and plots. As seen in the next sections, in an LDA scores plot each spectrum represents a point in LDA space and classes tend to form clusters. In these clusters the closeness between points implies spectral similarities while distance signifies class dissimilarities. Also, the plots can be used to identify chemical bonds distinguishing different treatment conditions.

In addition to IR spectroscopy and pattern recognition analysis, we investigated binding of Ag and TiO_2 NPs on Chelex®-100 and Metsorb™ using scanning electron microscope (SEM) to provide further evidence for retention of these NPs on the resins (see ESI for details).

2. Results and Discussion

Figure 3 and 4 show different regions of IR spectra obtained from Chelex®-100 and Metsorb™ after their exposure to silver and titanium dioxide nanoparticles and their ionic forms. As described earlier there are six different spectral classes for each resin, which three of them belong to silver samples; (1) Ag NPs, (2) Ag^+, (3) mixture of Ag NPs and Ag^+, and the other three belong to the titanium treatments; (4) TiO_2 NPs, (5) Ti^{4+}, (6) mixture of TiO_2 NPs and Ti^{4+}. As seen in these figures clear alterations are observed in different peak absorbance intensities or slight shifts in wavenumbers.

Both of the resins in this study are known for their abilities to bind cations because of their surface functional groups.[1,8,38] Figure 5 is a schematic representation of potential chemical bonds that may form between the binding agents and Ag^+ and Ti^{4+} cations. Chelex®-100 carboxyl and Metsorb™ surface titanium oxide/hydroxide hydroxyl groups can bind available Ag^+ and Ti^{4+} and irreversibly retain them. Deprotonation of carboxyl groups in Chelex®-100 occurs normally at pH ≈4.0, thus its overall surface charge in all the experiments with pH ≈ 5.8 is negative.[2,18] Details of Metsorb™ chemical structure is not fully disclosed, nevertheless we know that titanium oxides, TiO_2 and $Ti(OH)_4$ are the main components of this commercial product with TiO_2 dominating. Metsorb™ point of zero charge is pH ≈6.0[19,39] and has overall neutral to slightly positive surface charge under the current experimental conditions (pH ≈ 5.8). (see ESI for details).

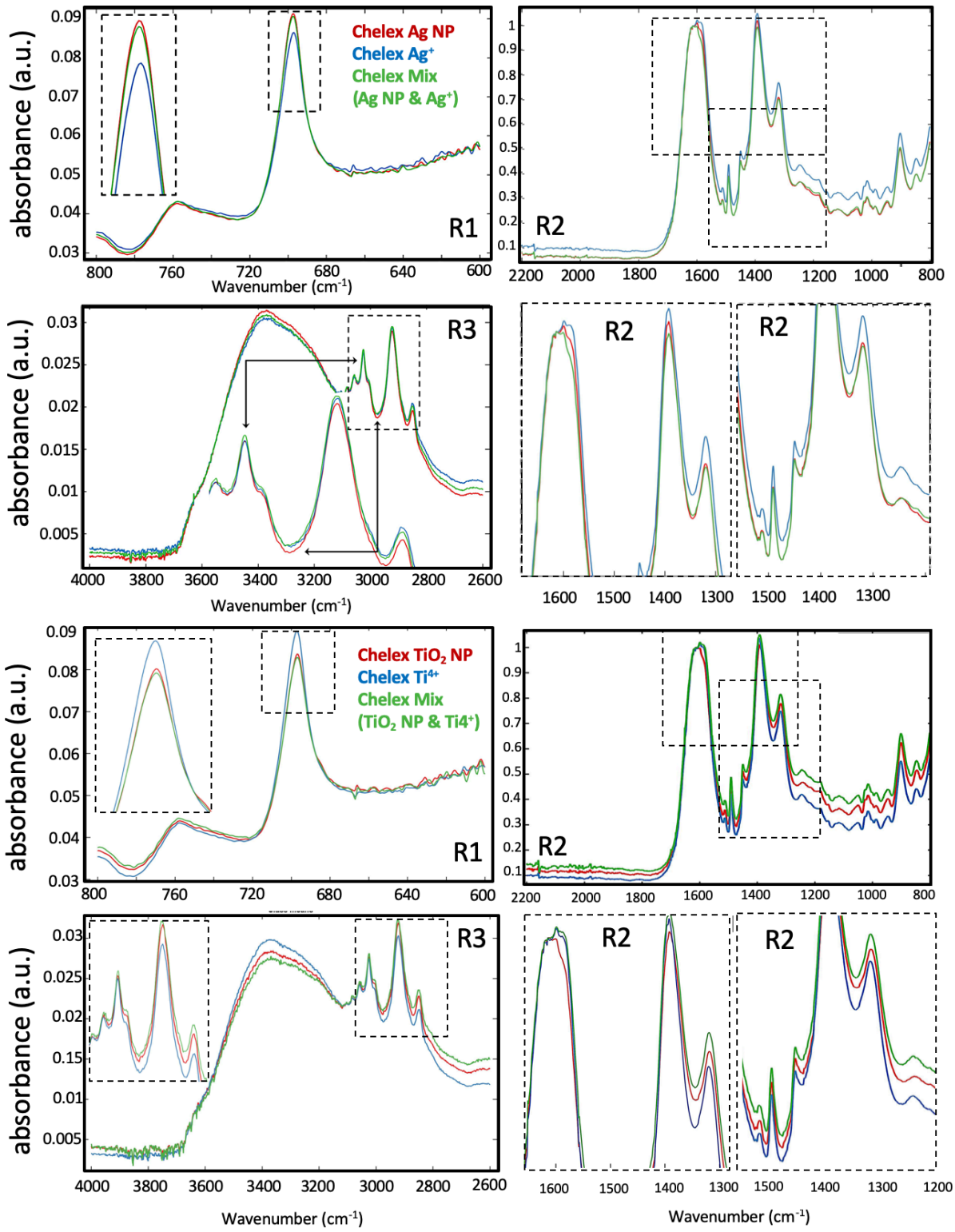


Figure 3. Comparison between derived infrared (IR) spectra for Chelex-100 exposed to the silver and titanium dioxide treatments. The IR spectra (600–4000cm−1) is divided into three spectral regions: Region 1 (600–800 cm−1), Region 2 (800–2200 cm−1), and region 3 (2600–4000 cm−1) for comparison purpose.

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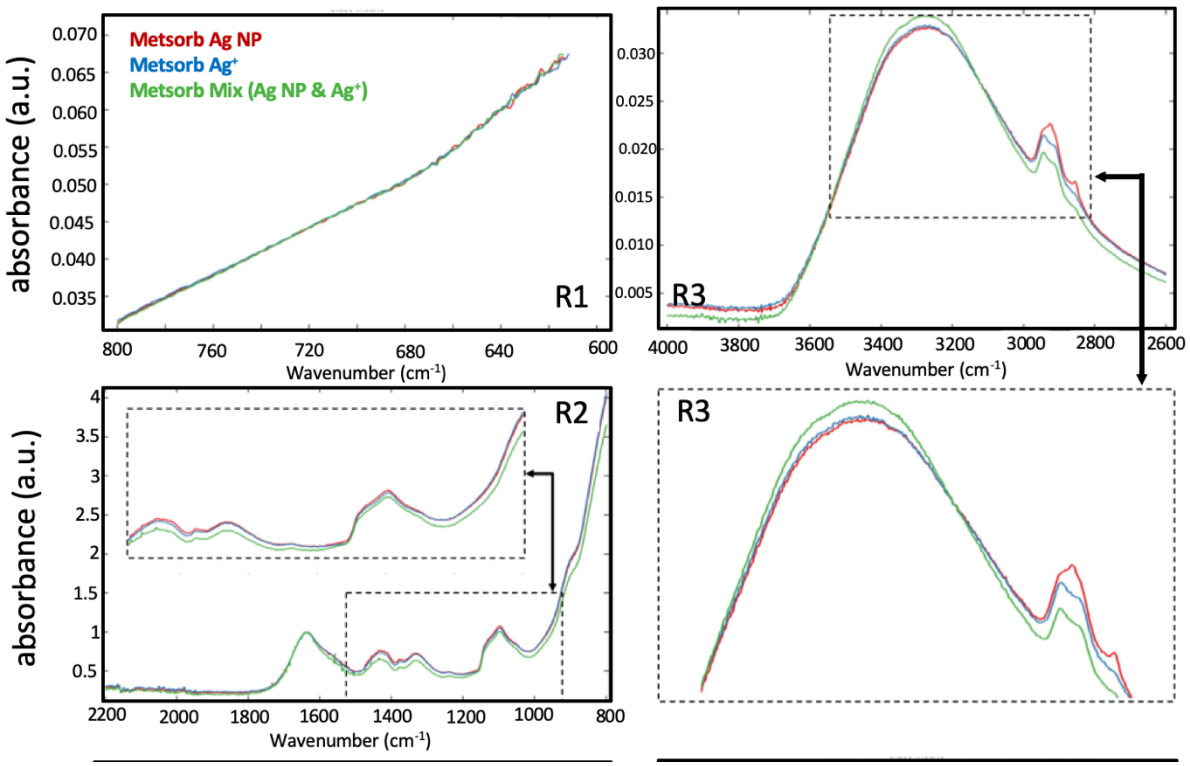

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Figure 4. Comparison between derived infrared (IR) spectra for Metsorb exposed to the silver nanoparticle and Ag+ treatments. The IR spectra (600–4000cm−1) is divided into three spectral regions: Region 1 (600–800 cm−1), Region 2 (800–2200 cm−1), and region 3 (2600–4000 cm−1) for comparison purpose (see ESI for IR spectroscopy results of Metsorb exposed to titanium dioxide nanoparticles)

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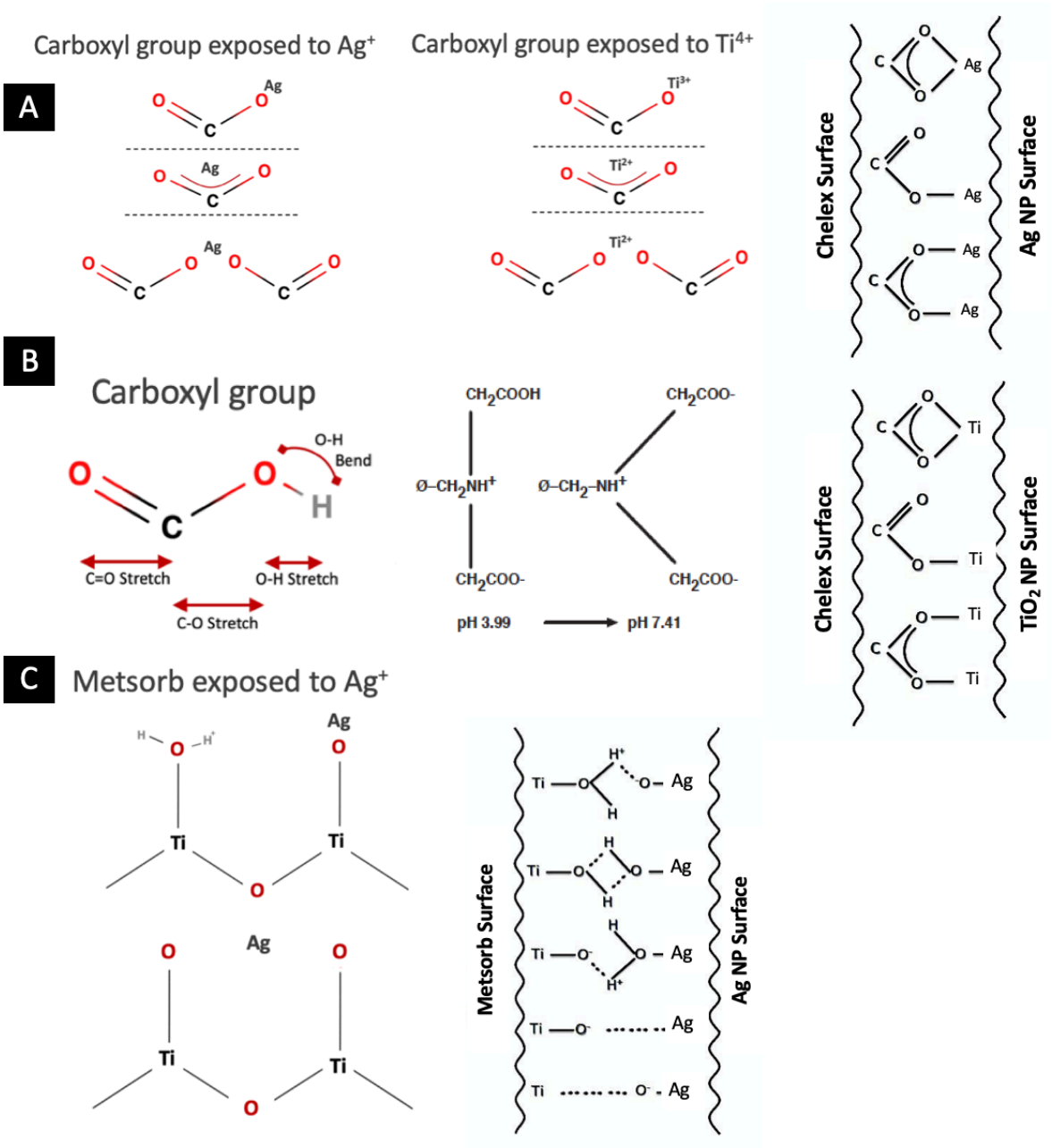

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Figure 5. Schematic representation of some of the major chemical bonds that could form between Chelex-100 carboxyl group with Ag^{+} and Ti^{4+} (A). Chemical structure of Chelex-100, its deprotonation and carboxyl group potential vibrations (B). some of the major chemical bonds that could form between Metsorb with Ag^{+} (C). Also, potential bond formation between the surfaces of Ag, TiO_2 NPs and Chelex-100 or Metsorb have been illustrated on the left side of this Figure.

240

241 It's worth noting that at any given pH, both protonated and deprotonated functional
242 groups exist at the same time on the resin surface, but their ratio is affected by the
243 concentration of protons. [26,40] Deprotonated surface functional groups on Chelex®-100,
244 carboxyl groups as seen in Figure 5, could bind Ag^{+} or Ti^{4+} directly. Also, two adjacent
245 carboxyl groups are able to chemisorb these cations, while hydroxyl and carbonyl groups
246 together could form complexes with them. Ag NP carries overall negative surface charge in
247 this experiment. Its surface includes oxidized, protonated and deprotonated sites that co-exist
248 at the same time on an Ag NP surface. Chemical bonds could form between protonated
249 surface hydroxyl groups of Ag NPs and deprotonated carboxyl. Other bindings between
250 structural Ag at the surface of Ag NP and carboxyl carbonyl and hydroxyl groups are also
251 possible. The same happens when Chelex®-100 is exposed to TiO_2 NPs, however, we know
252 that majority of hydroxyl groups formed on the surface of titanium dioxide nanoparticles are
253 protonated in this study, which could lead to stronger bond formation and retention of this
254 nanoparticle by Chelex®-100.

255 As illustrated in Figure 5, Metsorb™ surface deprotonated hydroxyl groups could form
256 chemical bonds with Ag^{+}. To lesser extent compatible bindings could happen between
257 Metsorb™ constituent chemicals as well as its surface functional groups with Ag NPs
258 (structural silver and protonated and deprotonated surface hydroxyl groups that form on this
259 nanoparticle surface).[1,26,27,40]

260 The afore-mentioned bonding characteristics should be reflected in the vibrational
261 changes of IR spectra of the samples.[27,41] Figure 3 and 4 represent IR spectra of these
262 chemicals in more details. In spectral region one (600-800 cm^{-1}), Chelex®-100 treatments
263 result in a notable alteration at ≈700 cm^{-1} with clear distinction (peak intensities and slight
264 shift in wavenumbers) between the different classes of spectra, but changes for peak at ≈760
265 cm^{-1} seems to be negligible. Interestingly, for Chelex®-100 silver and titanium treatments

show different patterns. At ≈700 cm^{-1} Ag NPs and the mixture of Ag NPs and Ag^{+} have stronger peak (more absorbance) compared to Ag^{+}. For the titanium samples, we have an opposite behavior and Chelex®-100 exposed to Ti^{4+} has a stronger peak compared to TiO_2 NP or mixture of Ti^{4+} and TiO_2 NPs. The peak at ≈760 cm^{-1} is broader, has considerably less strength compared to the one at ≈700 cm^{-1} and the observed changes are minor (because of the similarities of the peak intensities). These peaks can be assigned to C-H and N-H vibrations, respectively.[25,42]

The differences between theses peaks at ≈700 cm^{-1} could be stemmed from stronger polarity of chemical retention that happens between C-H and Ag NP. Higher number of the same chemical bonds between the resin and Ag NPs, could also contribute to the higher peak intensity. In contrary, it seems that for Chelex®-100 titanium treatments, it is Ti^{4+} that creates a stronger dipole moment and therefore stronger peak. For Metsorb™, the skewed broad peak that is extended to ≈800 cm^{-1} can be attributed to metal oxygen bond vibrations that are present in the titanium oxide in Metsorb™.[2,43]

In region two of the spectra, Chelex®-100 treatments show alterations at ≈900 cm^{-1} (C-H out-of-plane bending), ≈1330 cm^{-1} (O-C stretching), ≈1400 cm^{-1} (C-O-H in plane bending), ≈1430 cm^{-1} (O=C-O stretching of carboxylate group), ≈1500 cm^{-1} (N-H bending), and ≈1620 cm^{-1} (C=O stretching)[25,26,42]These changes suggest variations in strength of chemical bonds between oxygen (from C-O, O=C-O and C=O) and; Ag^{+} or Ti^{4+}, structural Ag of Ag NPs or Ti of TiO_2 NPs, or the surface hydroxyl groups formed on these nanoparticles. As described earlier Metsorb™ is mainly made of titanium oxide, however, has some impurities including ethanol (C_2H_4O) [2,19]. Bands in the second region of this resin's spectra at ≈1100 cm^{-1}, ≈1350 cm^{-1} and ≈1440 cm^{-1} are likely because of C-OH stretch, C-OH and O-H in-plane bend, respectively. [25,42,43] From these peaks it could be conferred that the surface hydroxyl groups are involved in bond formations with Ag^{+} and/or Ag NPs. The peak at ≈1650 cm^{-1} can be

attributed to the bending mode of H-O-H, raised from vibration of water or protonation of surface hydroxyl groups.[44]

For Chelex®-100 we see a broad O-H stretch peak in the region three, which spans from ≈2800 cm^{-1} to ≈3600 cm^{-1}, N-H stretching vibrations at ≈3400 cm^{-1} could also contribute to this peak. Symmetric and asymmetric stretching of C-H and =C-H stretching could be the reason for multiple bands seen at ≈2850 cm^{-1}, ≈2920 cm^{-1} and ≈3030 cm^{-1}.[27] For Metsorb™ spectra in region three O-H stretching can be identified as a broad peak at ≈3100-3600 cm^{-1} and the other peak, which appears at ≈2950 cm^{-1} can be assigned to Ti-OH bending.[43] Changes in intensities and shifts in these wavenumbers could be the result of the variations in electrostatic attractions between Ag^{+} and structural Ag, surface hydroxyl groups of Ag NPs with functional groups of these vibrational bands. [1,2] As mentioned earlier, in addition to stronger dipole moments between the bound entities, an increase in peak intensity could be attributed to a rise in the number of functional groups and surface sites associate with the chemical bonds that lead to the observed differences among the samples

Figure 6 and 7 are 3-D scores plots that exhibit PCA-LDA of absorbance spectra from Chelex®-100 and Metsorb™. Details of this computational analysis method are available in previous publications.[2,33–35] As seen multivariate analysis suggests that silver treatments and titanium samples form distinguishable and separate clusters of data in both Chelex®-100 and Metsorb™, which indicate that components of each of these groups have clear differences with each other. In these Figures the 3-D scatter plots separate analyzed (PCA-LDA) IR spectra based on their characteristic differences in their absorbance. [1,2,33] These analyses demonstrate that for derived spectra for Chelex®-100 all of the treatments ; (1) Ag NPs, (2) Ag^{+}, (3) mixture of Ag NPs and Ag^{+}, (4) TiO_2 NPs, (5) Ti^{4+}, (6) mixture of TiO_2 NPs and Ti^{4+}, are statistically different at the 95% confidence level (for more details see ESI). In other words, these six different spectral classes show statistically different absorbance. For

316 Metsorb™, as Figure 7 demonstrates, silver samples form three different clusters, which are
317 separated from each other. This segregation indicates that Metsorb™ spectra for these
318 treatments have different characteristics.

319 In this study the binding mechanisms of the two nanoparticles and their ionic forms to
320 Chelex®-100 and Metsorb™ are affected by the strength and number of chemical bonds
321 formed between them. For Ag^{+} and Ti^{4+} the resins are attracting readily available cations,
322 which have similar size to the binding sites on these resins, but for Ag and TiO_2 NPs they are
323 interacting with structural Ag and Ti or functional groups formed on their surfaces including
324 hydroxyls. In addition, Ag NPs carry over all negative surface charge at the pH of the
325 experiment while TiO_2 NPs have overall positive surface pH dependent charge in this
326 experiment (see ESI). Such surface charges influence electrostatic interactions that happen
327 between Chelex®-100 with negative surface charge and Metsorb™ with neutral to slightly
328 positive surface charge at the pH of the experiment (pH≈5.8). When Chelex®-100 or
329 Metsorb™ is exposed to Ag or TiO_2 NPs chemisorption occurs between two surfaces, with
330 impacts on the polarity of chemical bonds forming between the two entities as well as the
331 number of functional groups involved in sorption of the NPs, which both influence intensities
332 of the absorbance peaks in the IR spectra. This is compatible to what we observed in the IR
333 spectra of the treatments and deducted through computational analysis and pattern
334 recognition using PCA-LDA technique. It's worth highlighting that this research provides a
335 key finding with respect to diffusive gradients in thin-film (DGT) technique and confirms
336 that Chelex®-100 or Metsorb™ resins, which are of the most common binding materials in
337 DGT devices, are able to form chemical bonds with two of the most extensively used
338 nanomaterials in the world. Considering the well-established DGT technique for measuring
339 time-averaged concentrations of chemicals in the environment, the findings here could pave
340 the road for further development of DGT for measuring Ag and Ti nanoparticles in the

341 environment and helping us to improve our understanding of the fate and the behavior of
342 these materials in nature. In addition to ATR-FTIR spectroscopy and PCA-LDA analysis, we
343 have also used scanning electron microscope (SEM) imaging. The SEM images showed the
344 presence of Ag and TiO_2 nanoparticles on the Chelex®-100, however because of the physical
345 appearance and porous structure of Metsorb™ we couldn't identify these nanoparticles on the
346 surface of this resin. The results can be seen in electronic supplementary information (ESI).

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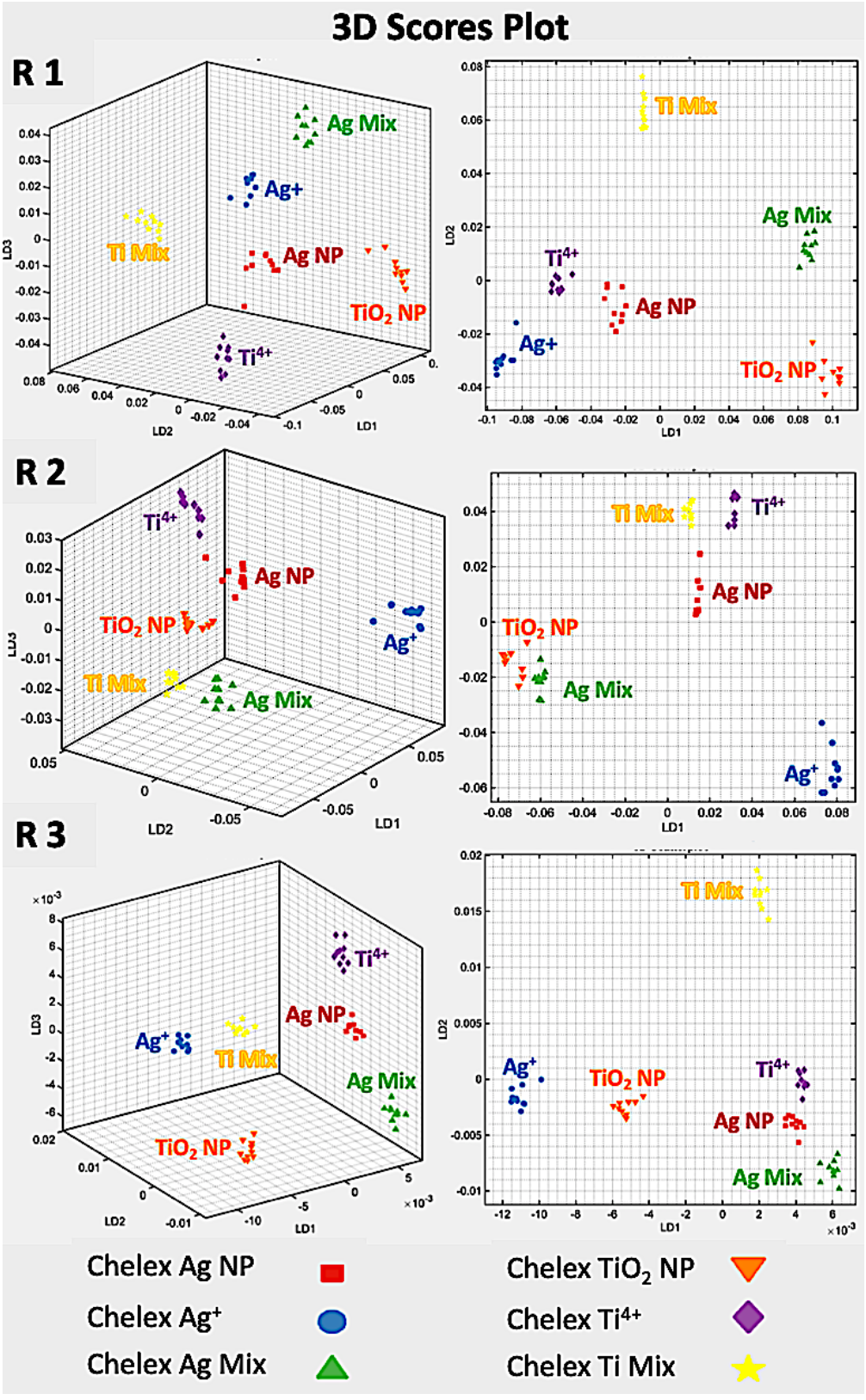

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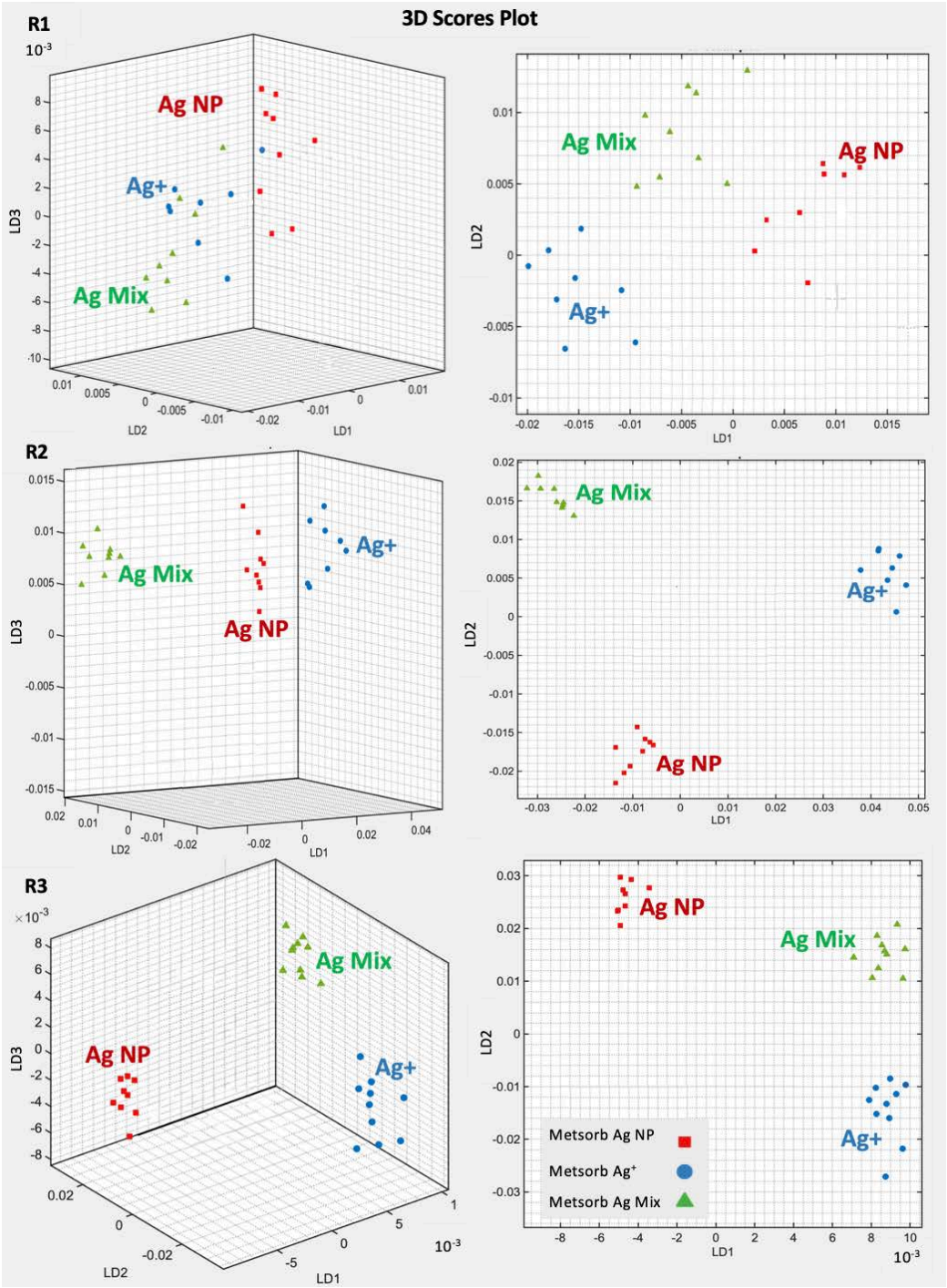
349 **Figure 6. Three-dimensional scores plot visualization of principal component analysis and linear**
350 **discriminant analysis (PCA-LDA) of Chelex-100 when exposed to Ag and TiO_2 treatments R1 (600–800 cm−1),**
351 **R2 (800–2200 cm− 1), and R3 (2600–4000 cm− 1), respectively, represent three spectral regions of the**
352 **spectra. Scales for X, Y, and Z-axis are arbitrary units. The graphs on the right show the top views of these**
353 **scores' plots.**



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357 **Figure 7. Three-dimensional scores plot visualization of principal component analysis and linear**
358 **discriminant analysis (PCA-LDA) of Metsorb when exposed to Ag treatments R1 (600–800 cm−1), R2**
359 **(800–2200 cm− 1), and R3 (2600–4000 cm− 1), respectively, represent three spectral regions of the spectra.**
360 **Scales for X, Y, and Z-axis are arbitrary units. The graphs on the right show the top views of these scores'**
361 **plots. (see ESI for PCA-LDA scores plot visualization of Metsorb exposed to titanium dioxide nanoparticles)**

362

1. Conclusions

This research shows that Ag and TiO_2 nanoparticles form chemical bonds with Chelex®-100 and Metsorb™, which are two commonly used binding resins in diffusive gradients in thin-film (DGT) method. It is reinforcing our previous findings about DGT potential for measuring engineered nanomaterials in soil and water[1,2]and is compatible with other studies.[9–13]This study also suggests that ATR-FTIR spectroscopy in combination with principal component analysis (PCA) and linear discriminating analysis (LDA), or other pattern recognition methods, could be developed to identify different types of chemicals retained by the binding resins. These give the confidence for using DGT technique measuring nanoparticles in the environment. However, further tests that include investigating diffusional characteristics of model Ag and TiO_2 in the DGT diffusive layers and the performance under a range of environmental conditions are required. of the further development of the DGT technique for measuring engineered nanomaterials in situ in soils and waters would allow us to have a better understanding of their behavior in the environment.

Supporting Information

Electronically supplementary information available: Surface potential of Ag and TiO_2 NPs used in this experiment. Derived infrared (IR) spectra for Metsorb exposed to the titanium dioxide nanoparticle treatments. Three-dimensional scores plot visualization of principal component analysis and linear discriminant analysis (PCA-LDA) of Metsorb exposed to TiO_2 treatments.Two-D visualization of principal component analysis and linear discriminant analysis (PCA-LDA) of Chelex®-100 and Metsorb™ when exposed to silver samples; (1) Ag NPs, (2) Ag^+, (3) mixture of Ag NPs and the titanium treatments; (4) TiO_2 NPs, (5) Ti^{4+}, (6)

mixture of TiO_2 NPs and Ti^{4+}. Scanning electron microscope (SEM) imaging of Chelex®-100 and Metsorb™ exposed to Ag and TiO_2 NPs.

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