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Covalent Attachment and Detachment by Reactive DESI of Sequence-Coded Polymer Taggants

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The use of sequence-defined polymers is an interesting emerging solution for materials identification and traceability. Indeed, a very large amount of identification sequences can be created using a limited alphabet of coded monomers. However, in all reported studies, sequence-defined taggants are usually included in a host material by noncovalent adsorption or entrapment, which may lead to leakage, aggregation, or degradation. To avoid these problems, sequence-defined polymers are covalently attached in the present work to the mesh of model materials, namely acrylamide hydrogels. To do so, sequence-coded polyurethanes containing a disulfide linker and a terminal methacrylamide moiety are synthesized by stepwise solid-phase synthesis. These methacrylamide macromonomers are afterward copolymerized with acrylamide and bisacrylamide in order to achieve cross-linked hydrogels containing covalently-bound polyurethane taggants. It is shown herein that these taggants can be selectively detached from the hydrogel mesh by reactive desorption electrospray ionization. Using dithiothreitol the disulfide linker that links the taggant to the gel can be selectively cleaved. Ultimately, the released taggants can be decoded by tandem mass spectrometry.

1. Introduction

Product identification and traceability are major issues in materials science. One facet of this topic is the spread of counterfeits, which is a worldwide problem as well as a threat to economy and consumer's health. Another important issue is materials recycling (e.g., commodity plastics), which requires reliable solutions for tracking and tracing batches. A wide variety of molecular and nanoscale technologies already exist for tracing and identifying materials.^[1] They rely on a variety of species such as peptides, polymers, liquid crystals, nanoparticles, and nanopatterned surfaces, and involve analytical methods such as visual detection, fluorescence imaging, calorimetry, and mass spectrometry. Among all these options, one interesting emerging trend is the use of sequence-defined polymers.^[2–3] In such polymers, monomer units are distributed in a precise order and therefore an information sequence can be stored in a

linear chain.^[4] Using a molecular alphabet based on two or more comonomers,^[5] a message (text, image, video, identification sequence, pin code, cryptography key) can be stored in a single molecule.^[6–13] This can be easily achieved, for example, with a natural sequence-defined polymer such as DNA, using its four biological nucleotides as a coding alphabet.^[4] However, DNA degrades relatively fast in solution or when heated.^[14] Thus, it cannot be used as such in many processing (e.g., in extrusion) and storage (e.g., in liquids) conditions. To be used as a reliable taggant, DNA has usually to be kept in a protecting medium, such as glass nanobeads.^[15] Alternatively, it has been recently evidenced that abiotic sequence-defined polymers can also be used for the same purpose.^[16–17] Indeed, using stepwise polymer synthesis,^[18–19] an information sequence can also be stored in a synthetic macromolecule. Moreover, man-made polymers are often less prone to thermal or chemical degradation than DNA. For example, it has been demonstrated that sequence-defined polyurethanes can be used as taggants in a wide variety of materials including 3D-printed polymer networks,^[16] solvent-casted polymer films,^[20] intraocular implants,^[21] and model in vivo implants.^[22] These polymer taggants are commercially available under the trademark name Poltag®.

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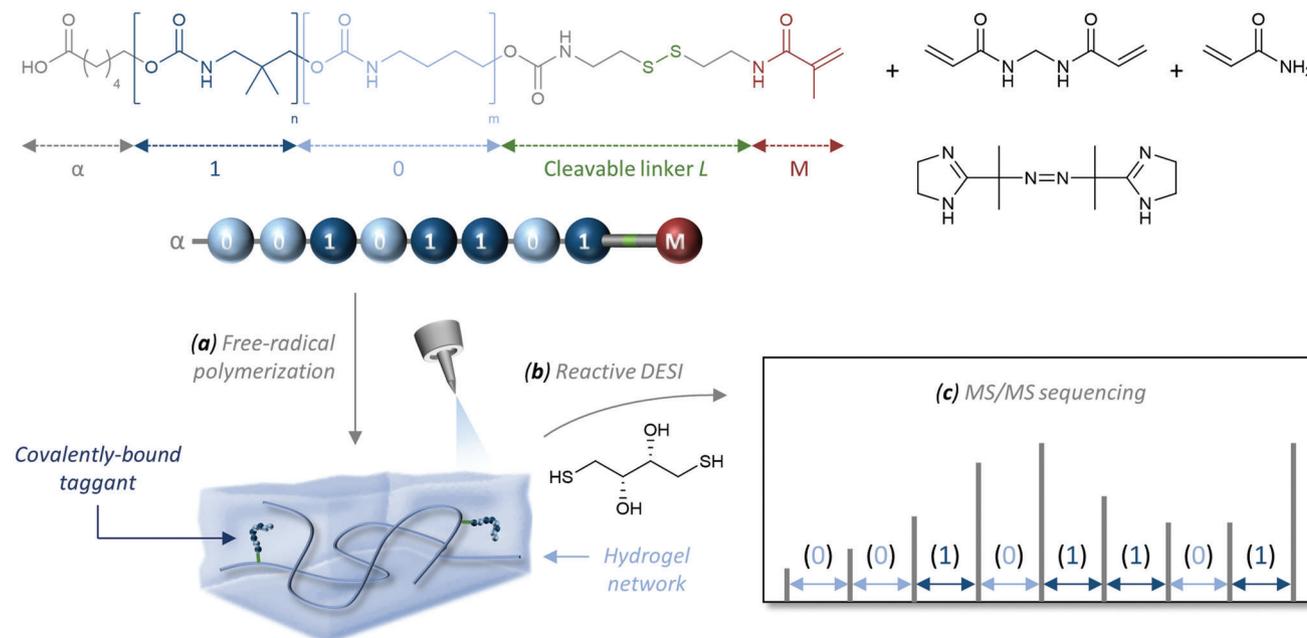


Figure 1. General concept investigated in the present work. a) A hydrogel containing covalently-attached polyurethane taggants is first synthesized by free radical copolymerization in aqueous medium. b) The gel is then subjected to reactive DESI-MS in the presence of dithiothreitol, and c) thus leading to the selective release of the taggants that can be ultimately decoded by MS/MS.

However, to label a specific material, polymer taggants are either physically adsorbed onto its surface or blended inside it. In other words, the taggants are only connected to the host material by weak noncovalent interactions and may therefore potentially diffuse out, segregate, or degrade. This can be a problem for some particular applications, e.g., in food industry, biomedical devices, and outdoor materials. It would be probably safer if sequence-coded taggants would be covalently attached to the host materials. Although covalent immobilization is by itself very easy to implement using any kind of reactive chemistry, it may also hinder the detection of the taggant. Indeed, the coded sequences of the taggant are generally deciphered by a sequencing technique,^[23] which requires in almost all cases that the taggant molecule is diffusible and extractable from the host materials. For instance, synthetic sequence-defined polymers are predominantly analyzed by tandem mass spectrometry (MS/MS),^[24] a technique that requires ionization and fragmentation of free macromolecules.

In this context, we describe herein a valid protocol for covalent attachment, selective detachment, and detection of sequence-coded polymer taggants (Figure 1). This strategy relies on reactive desorption electrospray ionization (DESI). DESI is an ambient ionization method, which makes use of high-velocity electrosprayed droplets to sample analytes from surfaces,^[25] as recently demonstrated for sequence-coded oligomers.^[26] Performing DESI in a reactive mode consists of adding reagents in the sprayed solution to induce specific ion/molecule reaction at the surface.^[27–29] Even though reactive DESI has been barely applied to synthetic polymers to date,^[30] this technique could potentially be used for detaching covalently-bound taggants and decoding them by MS/MS sequencing. This concept was applied herein to hydrogels, which is a very important class of

applied materials,^[31–32] but also probably the trickiest to label with sequence-coded polymers. Indeed, hydrogels are swollen polymeric networks (i.e., bicomponent materials made of polymer and water) and therefore physically adsorbed taggants may easily be washed out in standard conditions of use. In the present work, an optimal chemistry was developed for tagging hydrogels. Sequence-coded polyurethane macromonomers containing a polymerizable methacrylamide moiety and a disulfide linker (Figure 1) were covalently incorporated in hydrogel networks by free radical copolymerization. Furthermore, a reactive DESI protocol based on dithiothreitol (DTT) was developed in this work to reduce disulfide bonds.^[33] Consequently, the covalently-immobilized taggants can be detached on demand and analyzed by MS/MS.

2. Results and Discussion

Polyacrylamide gels were chosen as model hydrogels for the present study (Figure 1). They are obtained by free radical copolymerization of acrylamide and bisacrylamide and are used in several applications including polyacrylamide gel electrophoresis (PAGE)^[34–35] and oil extraction.^[36] The sequence-coded taggants studied for gels labeling were sequence-defined polyurethanes, which are prepared by stepwise orthogonal solid-phase synthesis as previously reported.^[16] In this work, the taggants were encoded with a binary molecular alphabet, in which 4-amino-1-butanol urethane (115 Da) and 3-amino-2,2-dimethyl-1-propanol urethane (129 Da) synthons represent bit-0 and bit-1, respectively. The advantage of this alphabet over previously reported ones is that carbamate interdistance is different in 0 and 1 repeat units, thus possibly reducing crystallization capability and enhancing solubility.^[37] It should be specified that this binary al-

Table 1. Polyurethane taggants that were studied in the present work.

Taggant	Sequence ^{a)}	$m/z^b)$	m/z_{th}
T1	α -1001-L-M	867.4199 ^{c)}	867.4202
T2	α -00101101-L-M	678.3562 ^{d)}	678.3560
T3	α -0011-L-M	884.4471 ^{e)}	884.4468
T4	α -00110101-L-M	678.3561 ^{d)}	678.3560

^{a)} The Greek letter α denotes the α -chain end. The numbers 0 and 1 represent coded monomer units. The letters L and M denote the cleavable disulfide linker and the polymerizable ω -chain end, respectively. See Figure 1 for details. ^{b)} Measured by positive-mode ESI-MS. ^{c)} Detected as $[M+H]^+$. ^{d)} Detected as $[M+2H]^{2+}$. ^{e)} Detected as $[M+NH_4]^+$.

phabet is used for academic demonstration only. For commercial applications, confidential alphabets are used. In order to attach the polyurethane taggants to the acrylamide networks and detach them by reactive DESI, their molecular structure was engineered as shown in Figure 1. The ω -terminus of sequence-defined polyurethanes can be easily modified by using a functional terminating agent in the final coupling step of the iterative synthesis.^[38] Here, the sequences were modified with *N*-methacryloylcystamine.^[39] This terminating agent contains a methacrylamide moiety that can be copolymerized with acrylamide and bisacrylamide, and a disulfide bond that can be reduced by reactive DESI. Although various types of chemistries can be considered for reactive DESI,^[28–29,40] disulfide groups were selected in the present study because they can survive radical polymerizations performed under mild reaction conditions.^[41–42]

Table 1 and Figures S1–S3 (Supporting Information) show the ESI-MS characterization of the polymerizable polyurethane taggants **T1–T4** that were studied in this work. They were all synthesized by stepwise solid-phase iterative synthesis.^[16] In the first attempts, the final step involving *N*-methacryloylcystamine proceeded in low yields and therefore the targeted methacrylamide macromonomers were only obtained as minor species. This situation was improved by using triethylamine instead of dry pyridine as a base in the terminal coupling step. In this case, the methacrylamide macromonomers (**Ti**) were isolated as dominating species, although the presence of symmetric diblocks (**Di**) as well as ω -amino-terminated oligomers (**Ni**) were observed in these samples, as evidenced by ESI-MS and confirmed by MS/MS sequencing (Figures S1–S4, Supporting Information). Since the two observed macromolecular impurities do not contain reactive double bonds and cannot be copolymerized with acrylamide monomers, the taggants **T1–T4** were used as such for hydrogel labeling. Indeed, rather than performing a time-consuming fractionation of macromolecular mixtures at this stage, the impurities were easily removed by solvent washing after network formation (vide infra).

The taggants macromonomers **T1–T4** were then incorporated into the corresponding acrylamide hydrogels **G1–G4** by free radical copolymerization. Since polyurethane taggants are poorly soluble in aqueous medium at room temperature, the gels were synthesized at 50 °C in a 1:2.5 v/v methanol/water mixture in the presence of the commercial azo initiator VA-044. The gels **G1–G4** were prepared in closed vials and therefore have a cylindrical shape with a diameter of 2.6 cm and a height of approximately 1 cm. Gentle stirring was used to avoid the forma-

tion of bubbles and to ensure the homogenous dispersion of the taggant in the gel's mesh. After synthesis, the gels were washed with water and methanol in order to remove excess reagents and impurities. Ultimately, they were dried under vacuum before DESI-MS analysis. The gels were then analyzed by DESI-MS. They were cut in thin lamellas with a surface of approximately 1 cm² that were fixed with double-sided tape on the sample plate and directly subjected to the DESI source, as illustrated in **Figure 2a,b**. Different pieces of each gel were analyzed in order to ensure the materials composition homogeneity as well as the reproducibility of the MS results. The gels were first analyzed in standard DESI conditions (i.e., in the absence of DTT) in order to screen the presence of noncovalently-attached coded oligomers. After standard washing procedures, it turned out that many samples still contained detectable traces of unbounded species, as shown in Figures S5–S6 (Supporting Information) for gels **G1** and **G2**. Although impurities such as symmetric diblocks and ω -amino-terminated oligomers can be easily distinguished from other oligomers in standard DESI conditions, it invalidates the interpretation of the reactive DESI measurements. Indeed, when subjected to reactive DESI in the presence of DTT, the covalently-attached taggant and the noncovalently-attached impurities both lead to the same thiol-terminated reaction products. Although such a signal was detected in all gels (Figures S5–S6, Supporting Information), it is therefore not possible to claim that it comes from covalently-attached taggants. In order to solve that problem, a more stringent solvent-washing procedure was established (Supporting Information). As shown in Figure S7a (Supporting Information) for gel **G4**, this procedure permitted to solve the aforementioned problem. Indeed, in these samples, no signal was observed in standard DESI analysis, thus confirming the absence of noncovalently-attached oligomers. Furthermore, when subjected to reactive DESI in the presence of DTT, a fragment with a terminal thiol was clearly detected at m/z 1210.7, thus confirming the presence of covalently-attached taggant oligomers in the gel. These results also confirm the relevance and efficiency of the disulfide/DTT combination for reactive DESI. To our knowledge, this is the first time that such a tailored sulfur-based linker is used in reactive DESI. Although the taggant was detected, it could not be decoded by MS/MS due to an insufficient abundance. Since the polyurethane taggant has a limited solubility in the copolymerization medium, it is possible that a large fraction of it is not incorporated in the polyacrylamide network. One possibility to bypass this problem is to use taggants with a shorter-chain length such as **T3**. Figure 2 shows the DESI-MS results obtained in the presence and in the absence of DTT for gel **G3** (Figure 2c). In standard DESI conditions, no signal due to noncovalently-adsorbed species was observed, whereas in reactive DESI the expected thiol-terminated oligomer was detected in all-studied fragments of **G3**. These results confirm that the covalently-attached taggant is well-distributed within the network, even though some variations of ion intensities were noted. Furthermore, the released thiol-terminated taggant could be sequenced by MS/MS, as shown in Figure 2e.

3. Conclusion

In summary, it is shown herein that sequence-defined polymer taggants can be covalently attached to a host material, while still

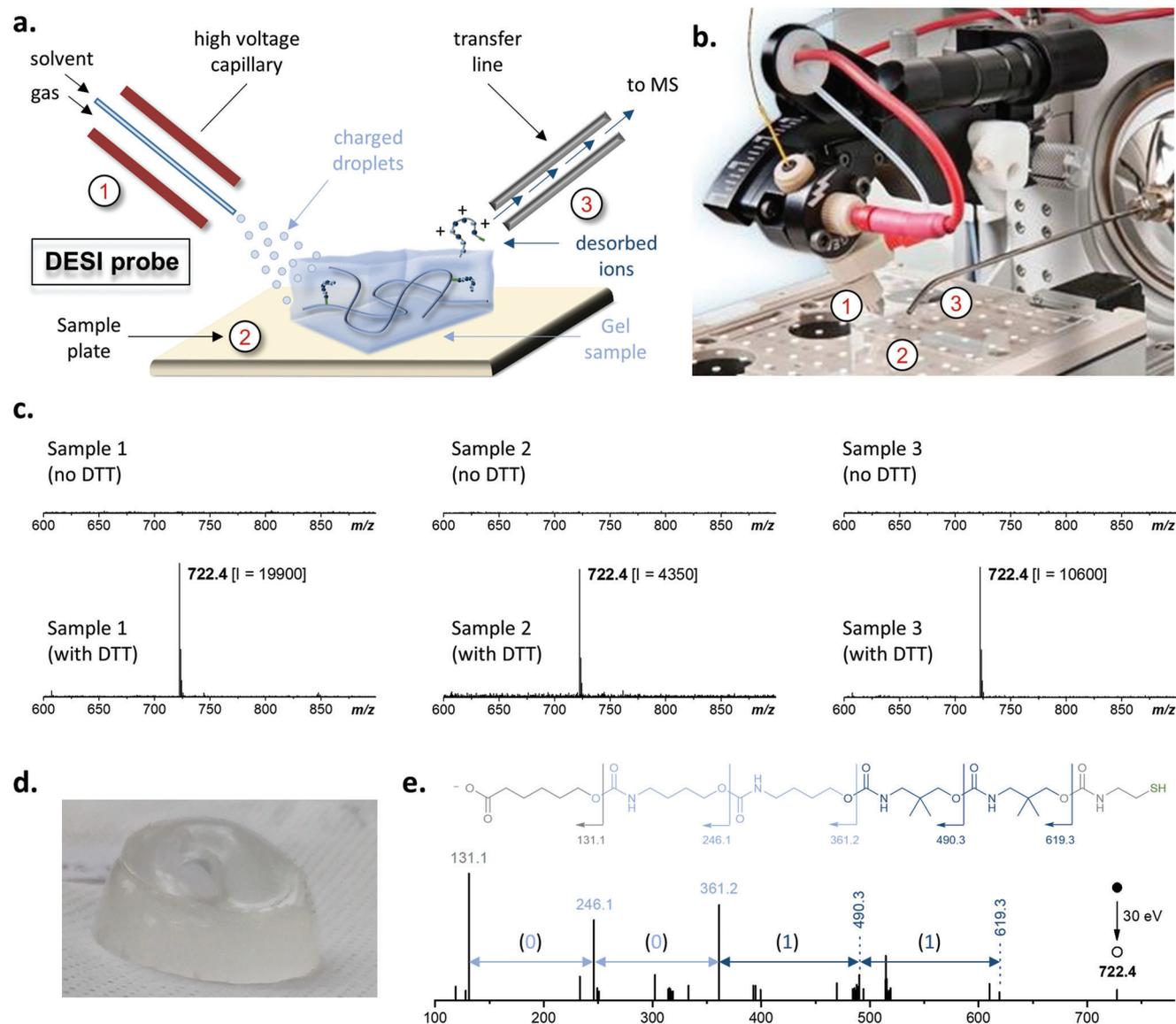


Figure 2. a) Schematic representation and b) picture of the DESI probe used for gel analysis. c) Negative ion mode DESI-MS spectra recorded for three different fractions of the gel **G3** after performing DESI in the absence (top) or in the presence (bottom) of DTT. Absolute intensity measured for the released thiol-terminated species at m/z 722.4 is indicated into brackets. d) Picture of the tagged polyacrylamide hydrogel **G3**. e) MS/MS spectrum recorded for the deprotonated taggant **T3** after selective gel detachment.

being accessible for MS/MS identification. Indeed, using a tailored disulfide linker between the taggants and the host matrix, it is possible to selectively cleave the taggants by reactive DESI and to decode their sequence. These results emphasize for the first time the great relevance of reactive DESI for materials identification and traceability. This approach is particularly appealing for anti-counterfeiting applications because it can be directly applied to the surface of a host material and does not necessitate time-consuming solvent extraction procedures. It shall also be reminded that the hydrogels studied herein were chosen on purpose as tricky soft materials. Thus, the reactive DESI-MS strategy described herein can most probably be extended to other types of materials (e.g., solid surfaces) and in that case might even be easier to apply. Overall, the results of this publication evidence even

further the relevance of sequence-controlled polymers for identification technologies.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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The first paragraph in the introduction was added on July 25, 2022, following a technical error wherein this was missing. The editorial office apologizes for any inconvenience caused.

Conflict of Interest

M.M., A.A.O. and J.M. are employees of the company Polysecure GmbH that commercializes the product Poltag®. J.F.L. and L.C. are inventors of a related patent.

Data Availability Statement

The data that support the findings of this study are available in the Supporting Information of this article.

Keywords

anti-counterfeiting technologies, desorption electrospray ionization, materials traceability, secure materials, sequence-controlled polymers

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