In situ surface functionalization of plasticized poly(vinyl chloride) membranes by ‘click chemistry’†

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We report here for the first time a universal method to achieve a covalent surface modification of plasticized poly(vinyl chloride) (PVC). A copper(i)-catalyzed azide–alkyne cycloaddition (‘click chemistry’) is performed on plasticized PVC containing partial azide substitutions. This surface modification is performed under mild conditions after membrane casting and is likely to be generally applicable to electrochemical and optical sensors. The concept is illustrated by attaching fluorescein and sulfonated Nile blue derivatives, as well as tetaethylene glycol to the membrane surface. Characterization by confocal microscopy, ATR-IR, QCM, UV/Vis spectroscopy and pulsed chronopotentiometry supports the surface modification procedure. As an initial example of practical utility, tetaethylene glycol modification is shown to significantly reduce surface adsorption by albumin, as evidenced by QCM and electrochemical experiments.

Introduction

Poly(vinyl chloride) (PVC) is used as a polymer matrix of choice in ion-selective electrodes (ISEs), which are well-established tools in the clinical analysis of ions such as H⁺, Na⁺ and K⁺ in blood samples. Its mechanical properties, excellent compatibility with electroactive additives, as well as low price and facile membrane preparation make it a polymer of choice for most polymer membrane ion-selective electrodes and also many optical ion sensors. In view of this, the surface modification of plasticized PVC membranes is especially interesting for tuning properties at the membrane–solution interface of ion sensors. The surface modification of PVC may help, for instance, in the design of chemical sensors with improved biocompatibility. Moreover, receptors or enzymes suitable for biosensor design can be covalently attached to the sensor surface.

Upon blood contact, the adsorption of proteins like albumin and fibrinogen takes place, followed by platelet adhesion and activation, resulting in blood coagulation and subsequent sensor failure. Meyerhoff’s group proposed a NO-releasing polymer with improved blood compatibility and attractive analytical characteristics. In another approach the well-established anticoagulant heparin was grafted onto a polymeric sensing surface. This reaction is characterized by high yields, compatibility with a wide variety of functional groups and very mild reaction conditions. The most important feature of this reaction, in view of modifying plasticized PVC membranes, is the possibility of using aqueous solutions at room temperature as the reaction medium. Water as a solvent prevents polymer dissolution and leaching of plasticizer and other membrane components during the reaction, which is a problem when using organic solvents.

Copper(i)-catalyzed azide–alkyne cycloaddition (CuAAC) is a very versatile method employed with success in many polymer modifications, both in bulk and on the surface. This reaction is characterized by high yields, compatibility with a wide variety of functional groups and very mild reaction conditions.

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PVC is a good candidate for ‘click’ modification as the chloride groups may be transformed into azide groups by nucleophilic substitution.

† Electronic supplementary information (ESI) available: Syntheses details, photos of the Nile blue-modified membrane surfaces and cross-sections, 3D projection of fluorescein-modified membrane sandwiched with blank membranes, additional electrochemical figures. See DOI: 10.1039/c2jm31118f

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‡ We reported recently on the bulk modification of PVC with ferrocene groups, which acted as an ion-to-electron transducer in solid contact ISEs, with much improved properties compared to PVC underlain with conducting polymers. Here we report on the application of dyes as visual probes of plasticized PVC surface modification, and modification with...
tetraethylene glycol (TEG) as an example leading to increased biocompatibility of a PVC membrane.

**Experimental**

**Reagents, materials and equipment**

High molecular weight poly(vinyl chloride) (PVC), tetrade-cylammonium tetrakis(4-chlorophenyl)borate (ETH 500), bis(2-ethylhexyly) sebacate (DOS), sodium ionophore X, sodium chloride, magnesium nitrate, tris(hydroxymethyl)methylamine (TRIS), bovine serum albumin (BSA), anhydrous tetrabromo-furan (THF), together with all reagents and solvents used in the synthesis were purchased from Sigma Aldrich and used without further purification. Aqueous solutions were prepared by dissolving the appropriate salts in Milli-Q-purified distilled water. QCM experiments were carried out with a Q-Sense E4 (Gothenburg, Sweden) instrument in a flow through cell. IR spectra were recorded using a Perkin-Elmer Spectrum One spectrometer. UV-Vis spectra were recorded on a Specord 250 spectrometer (Analytich Jena, Jena, Germany), using unmodified membranes as blanks. Confocal images were recorded on a Zeiss LSM 510 Meta Laser Scanning Microscope (Carl Zeiss, Inc., Germany). All electrochemical measurements were performed with a three-electrode configuration using a large platinum rod as a counter electrode, a double-junction Ag/AgCl/sat. KCl/l M LiOAc as a reference electrode (Mettler-Toledo AG, Schwerzenbach, Switzerland) and the TEG-modified PVC membrane mounted in an Ostec body (Oesch Sensortecchnology, Sargans, Switzerland) as a working electrode. Measurements were performed at ambient temperature with an Autolab PGSTAT128N (Metrohm Autolab, Utrecht, The Netherlands) using Nova 1.6 software.

**Membrane preparation and modification**

Membranes used for modification with Nile blue were prepared by dissolving 66 mg of N2PVC and 134 mg of DOS in 1 ml of THF and then casting in glass rings with an inner diameter of 2.2 cm.

The membrane cocktail for TEG modification was prepared by dissolving 60 mg of N2PVC, 120 mg of DOS, 20 mg of ETH 500 and 5.9 mg of sodium ionophore X in 1 ml of THF. A mother membrane for modification was prepared by drop-casting the cocktail in a glass ring, 2.2 cm in diameter, and letting the THF evaporate overnight under ambient conditions.

The ‘click reaction’ mixture was prepared by mixing solutions of 7.7 mg (0.030 mmol) of CuSO4·5H2O and 27.8 mg (0.158 mmol) of ascorbic acid in 0.5 ml of water or water–THF mixture (4% of THF v/v) and 1.7 mg (0.007 mmol) of clickable TEG, or 1.7 mg (0.002 mmol) of clickable Nile blue. The glass ring was then covered with parafilm to prevent evaporation and left overnight. Afterwards, the reaction mixture was removed from the glass ring and the membrane was washed repeatedly with water. Alternatively, the unmodified membrane was cut, mounted in a commercial Ostec body (Ostec, Sargans, Switzerland) and the tip of the electrode was subsequently immersed in the reaction mixture.

**Electrochemical measurements**

Membranes of 7 mm in diameter were cut from the modified membranes and installed in Ostec bodies containing 10 mM NaCl as an inner electrolyte. The electrodes were conditioned overnight in 10 mM NaCl. A previously reported25 multi-pulse electrochemical protocol was used for the measurements. The procedure consisted of three pulses. The first pulse of –9 μA was applied for 0.5 s followed by a zero current pulse for another 0.5 s and finally a regeneration pulse was applied for 25 s at 0 V. Potentials found at the end of the second pulse were used as data points. All measurements were performed in 10 mM Mg(NO3)2 + 1 mM TRIS buffer (pH = 7.4) background.

**Quartz crystal microbalance measurements**

QCM measurements were carried out using gold-coated quartz crystals spin-coated with PVC or N2PVC membranes. The N2PVC-covered crystal was then modified with TEG using the procedure described above. Experiments were performed in a flow through cell with 1 mM TRIS buffer solution (pH = 7.4) pumped into the cell for 3 min before and after 40 g l⁻¹ BSA solution.

**Results and discussion**

PVC was functionalized by substitution of 8 mol% of its chloride groups by azide moieties using a previously described procedure.24 For this purpose a membrane cocktail with a classic composition of 2 : 1 plasticizer (DOS) to polymer ratio was prepared and the membrane was formed by solvent-casting from THF. In order to visualize the localization of the subsequent surface modification, ‘click chemistry’ on the cast plasticized membranes was performed with a fluorescent compound. The clickable fluorescein and Nile blue derivatives 7a, 7b and 9 were synthesized for this purpose using the pathways shown in Scheme 1. One set of membranes was treated with a reaction mixture containing clickable dye and catalyst system (CuSO4–ascorbic acid) in water, and another with additional 4% (v/v) of THF. This addition of THF was explored to help the reagents penetrate the outer layer of the membrane while otherwise not affecting the polymeric membrane. The blanks were membranes treated with analogous solutions but without copper catalyst.

All membranes (with and without catalyst) that were treated with fluorescein 7a exhibited fluorescence, even after prolonged washing, suggesting that its structure is sufficiently lipophilic to diffuse into the polymer membrane. To best visualize the distribution of dye and to exclude surface scattering and optical interference effects that could otherwise mask the results, the treated membranes were sandwiched with a second membrane prepared from unmodified PVC, and the fluorescence distribution was observed as a function of time with confocal microscopy (see Fig. 1). Indeed, penetration of fluorescein into the unmodified portion of the sandwich can be observed, confirming that unreacted dye partitioned into the membrane during prior incubation. Note that the region of highest fluorescence remained on the surface of the membrane as evidenced by a sharp boundary between the two segments. Negligible blurring of this boundary region with time was observed, confirming a covalent surface modification. In the case of the blank experiment (Fig. 1c), dye fluorescence was found to equilibrate more homogeneously between the two segments without a strong membrane-internal fluorescent boundary. In order to omit
spontaneous partitioning of the dye during surface modification, the fluorescein 7a structure was further altered. The more hydrophilic PEG 600 (which corresponds to about 12 monomeric units) was introduced as a linker instead of tetraethylene glycol, giving fluorescein 7b. As shown in Fig. 2, increasing the length of the linker prevented the diffusion of dye into the membrane. After 72 h no fluorescence was detected in the blank membrane on top of the modified sample, suggesting that there is no free dye or that there is no spontaneous diffusion of fluorescein 7b into plasticized PVC. Note that N3PVC membranes also showed a certain degree of autofluorescence. This background fluorescence was also observed in the sample treated with fluorescein 7b but without catalyst, confirming that 7b does not spontaneously diffuse into the membrane. In addition to the fluorescein experiments, the sulfonated Nile blue derivative 9 was synthesized. A clear difference between the Nile blue sample with and without catalyst was observed, particularly in the case of the sample with THF addition (see ESI†). Nile blue 9 is indeed fluorescent in solution but measurement of the modified surface showed no fluorescence, even though a surface coloration was visible with the naked eye. Nile blue 9 exhibits strong absorbance in the visible region of the spectrum, and visualization of the surface ‘click chemistry’ was achieved by UV-VIS spectroscopy.

The appearance of membranes from blank experiments did not change, confirming the high hydrophilicity of the dye, while membranes after contact with the reaction mixture became bluish, with a more intense color in the case of modification in water–THF (see ESI, Fig. S1†). UV-VIS measurements confirmed this observation (Fig. S2†). To examine the penetration of the dye into the sample, thin slices of the membranes were
cut and microscope images of the cross-sections were taken (see Fig. S3†). Dye coloration was observed only in a thin surface layer of the sample and indeed no diffusion into the bulk membrane took place. Fig. 3 presents ATR-FTIR spectra of membranes modified with Nile blue 9, with and without addition of THF to the reaction. The light beam penetrates here only a very thin layer of the sample. The most distinctive feature of these spectra is the peak at 2100 cm⁻¹, corresponding to the azide group stretching vibrations. For surface-modified samples a new peak appeared at 1595 cm⁻¹ which can be attributed to the triazole moiety formed in the reaction (Fig. 3, inset).²⁶ To confirm the origin of this peak, a membrane with Nile blue 9 dissolved in its bulk was prepared. This signal was more intense in the case of a membrane modified with addition of THF than in water alone and was indeed absent in the sample with 9 dissolved in the matrix (Fig. 3, inset). Furthermore, no triazole signal was observed on the bottom side of the modified membranes. ATR-FTIR spectroscopy was also used to assess the time needed to complete the process. Several membrane samples were treated with reaction mixture for various times and the azide peak was used as a probe to monitor the reaction progress (Fig. S4†). These data suggest that the reaction is complete in about 100 min, although the peak does not disappear completely. This may be caused by the penetration depth of the ATR-FTIR beam being larger than the reaction depth, or by inaccessible azide groups in the surface layer.

In view of offering a solution to improve the biocompatibility of PVC membranes in analytical applications using this methodology, we modified the surface with the hydrophilic, clickable tetraethylene glycol 2a (TEG 2a). This linker was attached to a N₃PVC-based sodium-selective membrane using the CuAAC reaction in water–THF. A mother membrane of approximately 100 μm in thickness was cast and subsequently modified with TEG 2a in a glass ring. Albumin was chosen as a model protein because it is one of the most abundant blood proteins with a concentration of ca. 40 g l⁻¹ and prone to adsorb onto PVC-based membranes.²⁷,²⁸

Quartz crystal microbalance and electrochemical measurements were used to study the surface properties of these modified membranes. The electrochemical protocol made use of a constant current pulse to drive sodium ions into the membrane, followed by a period at zero current to measure the membrane potential. As established previously with polyelectrolyte membranes²⁹ and surface-confined biotinylated films,²⁵ this protocol can reveal the existence of a surface layer that acts as
a barrier to mass transport and extraction. When a barrier is present, the more abundant background ion (magnesium) is extracted instead, resulting in a potential change.

The electrodes were tested first in the absence of albumin in the sample solution. Modification with TEG 2a increased the detection limit of the electrode to some extent, although it is without practical importance (Fig. S5†).

Subsequently, albumin was successively added to a 1 mM NaCl background. Since albumin is available as a sodium salt, its addition should cause a constant, positive potential change with increasing albumin concentration. Instead, the observed potential decreases for PVC and N₃PVC at low albumin concentration (Fig. S6†). As mentioned above, this is explained with an attenuated ion transfer. For membranes based on surface-bound TEG-PVC this effect was much smaller.

After BSA addition the electrodes were washed and the sodium additions were repeated. The obtained calibration curves are presented in Fig. 4. The results obtained for PVC and N₃PVC electrodes were found to be shifted to higher concentrations by about 0.5 orders of magnitude compared to those obtained for fresh electrodes. This indicates that residual albumin was adsorbed on the membrane surfaces. In the case of TEG 2a the shift was negligible and in agreement with the results obtained during albumin additions. Moreover, the sodium calibration curves obtained with a N₃PVC membrane treated with TEG 2a without catalyst, before and after contact with BSA, were in agreement with the results from an unmodified N₃PVC membrane. This result shows that TEG 2a diffusion into the membrane (if any) does not influence the surface properties.

Quartz crystal microbalance characterization was used as a reference method to confirm the electrochemical results. Gold-covered crystals were spin-coated with PVC and N₃PVC, the latter of which was then modified with TEG 2a, and investigated in a flow cell. 1 mM TRIS buffer was pumped into the cell before and after exposure of the membrane to 40 mg ml⁻¹ BSA in the same buffer. As shown in Fig. 5, a significant decrease of about 65 Hz in the measured frequency was observed for unmodified PVC electrodes in the presence of 40 mg ml⁻¹ BSA in 1 mM TRIS pH 7.4 + 10 mM Mg(NO₃)₂ background. Before and after BSA addition samples were washed with background solution, suggesting substantial albumin adsorption at the membrane surface. The subsequent buffer treatment gave a frequency difference before and after albumin exposure of 30 Hz and confirms its affinity to the membrane surface. For N₃PVC-based membranes, the frequency change was somewhat smaller at 15 Hz. For TEG-PVC, the frequency difference before and after exposure to albumin was insignificant. These results are in agreement with the electrochemical data and suggest that plasticized PVC membranes can be chemically modified by a ‘click chemistry’ approach and that the TEG-PVC layer results in a drastically diminished adsorption of BSA onto the membrane.

Conclusions
Plasticized PVC membrane surfaces were successfully modified for the first time using a CuAAC reaction. Application of clickable dyes revealed an important role of their hydrophilicity in the visualization of surface modification. The more lipophilic fluorescein derivative 7a diffused deep into the membrane while the more hydrophilic 7b and Nile blue derivative were confined to the surface. This shows that the careful choice of the structure of a clickable molecule is vital to avoid uncontrollable diffusion. Confocal microscopy, together with UV-VIS and ATR-FTIR spectroscopy measurements confirmed the modification of the membrane surfaces. Experiments showed that addition of THF to the reaction mixture gives improved yields compared to water alone, likely because of a better penetration of the reactants into the outermost layers of the membrane. Applying this method for the preparation of PVC membranes with hydrophilic surfaces using TEG 2a allowed one to obtain more biocompatible electrode surfaces. Electrochemical experiments using a current pulse protocol showed that BSA adhesion on the membrane surface was reduced compared to unmodified PVC. This was confirmed by QCM measurements, where adsorbed protein could be easily washed off from TEG 2a-modified surfaces, whereas BSA was strongly retained on unmodified PVC membranes. The successful attachment of three structurally different compounds using essentially the same, mild reaction conditions on prepared membranes, suggesting substantial albumin adsorption at the membrane surface.
membranes contradicts previous findings" and opens the way for tailor-made surfaces that will suit the intended applications of electrochemical and optical sensors made from this sensing material.

Notes and references