Detection of Alkylating Agents using Electrical and Mechanical Means

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Abstract. Alkylating agents are reactive molecules having at least one polar bond between a carbon atom and a good leaving group. These often simple molecules are frequently used in organic synthesis, as sterilizing agents in agriculture and even as anticancer agents in medicine. Unfortunately, for over a century, some of the highly reactive alkylating agents are also being used as blister chemical warfare agents. Being relatively simple to make, the risk is that these will be applied by terrorists as poor people warfare agents. The detection and identification of such alkylating agents is not a simple task because of their high reactivity and simple structure of the reactive site. Here we report on new approaches to the detection and identification of such alkylating agents using electrical (organic field effect transistors) and mechanical (microcantilevers) means.

1. Introduction

Alkylating agents, such as methyl iodide, dichloromethane and epichlorohydrin are commonly used in organic synthesis as reagents and solvents. Similar alkylating agents, such as methyl bromide, are still used as soil sterilizers,¹ while nitrogen mustards are being used as anticancer drugs.² Alkylating agents are also dangerous byproducts of the water purification process.³ Unfortunately, due to their ease of production and storage, similar systems are also used as chemical warfare agents.⁴ Owing to their ability to react with nucleophiles in our body, most of these materials are toxic, carcinogenic and/or mutagenic.⁵ The combination of simple synthesis with aggressive reaction with biological tissues makes many alkylating agents perfect potential “poor people” chemical warfare agents and thus a perfect terror inflicting weapon. Therefore, there is a clear need for simple, sensitive and informative tools for the detection and identification of such agents, especially in the gas phase.

Tailoring selective receptors for small and reactive molecules is a non trivial and challenging goal. Additionally, it is much easier to synthesize a new toxic alkylating agent than to tailor a selective receptor for its detection, rendering this approach inefficient. Previous attempts to address this challenge resulted with two basic approaches. The first approach gave up selectivity, detecting all alkylating agents in a non specific way, relying on a color change of a nucleophile upon reacting with an alkylating agent.⁶ The
second approach focused on a limited family of alkylators by detecting a specific mass or the presence of a specific atom it contains (for example the sulfur atom in sulfur-based mustard gas). One possible solution to the detection of alkylating agents could be the use of a nucleophile as a trap for alkylating agents as an efficient nucleophile, such as an amine group, is expected to react with all the reactive alkylating agents as they are essentially electrophiles, Scheme 1. This reaction guarantees very high sensitivity, as the binding is no longer reversible.

![Nucleophile Alkylating Agent](image)

**Scheme 1**: General equation for the reaction of a nucleophile (Nu:) with an alkylating agent.

The reaction of the nucleophile with the alkylating agent needs to be reported and this may be achieved through one on a wealth of methods developed along the years. For example, we have recently reported on using luminescence turn-on as well as luminescence shifts for the detection and identification of different alkylating agents.

Here we report on the coupling of the reaction between a nucleophile and alkylating agents to electrical (organic field effect transistors, OFETs) and mechanical (microcantilevers) signal transducers for the detection, risk assessment and identification of alkylating agents. The three complimentary methods demonstrate the potential of using such reactions for the detection of reactive substances.

### 2. Results and Discussion

#### 2.1 Detecting alkylating agents using organic field effect transistors

Coupling of a nucleophile to an organic semiconductor was achieved by co-polymerizing 4-[2,5-bis (2-thienyl)-3-thienyl] pyridine, 1, with 3-hexylthiophene, 2, to obtain co-polymer 3, Scheme 2.

![Scheme 2](image)

**Scheme 2**: Preparation of co-polymer 3. i: Ba(OH)$_2$.8H$_2$O + Pd[Ph$_3$P]$_4$; ii: NBS in acetic acid; iii: Pd[Ph$_3$P]$_4$; iv: FeCl$_3$ in chloroform; v: H$_2$N-NH$_2$.

3-(4-pyridyl)-thiophene, 6, was prepared in 75% yield using palladium catalyzed cross coupling of thiophene-3-boronic acid, 4, with 4-bromo pyridine, 5. 6 was di-brominated at the 2 and 5 positions using NBS in acetic acid, affording 2,5-dibromo-3-(4-pyridyl)-thiophene, 7, in 60% yield. Palladium catalyzed cross coupling of 7 with two equivalents of 2-(tri-n-butyl stannyl) thiphene, 8, afforded 4-[2,5-bis (2-thienyl)-3-thienyl] pyridine, 1, in 80% yield. Co-polymer 3 was obtained as a bright red solid, Mn=37000, Mw=97000 (GPC), in 17% yield by co-polymerization of a 1:10 solution of 1 and 2 in chloroform in the
presence of FeCl$_3$. NMR characterization reveals that co-polymer 3 is composed of $2,3_n$ where $n=0.95\pm0.005$ and $m=0.05\pm0.005$.

Co-polymer 3 reacts with alkylating agents according to Scheme 3 both in solutions and as a film when treated with their vapors.

\[ \text{Scheme 3: Reaction between co-polymer 3 and an alkylating agent.} \]

Co-polymer 3 is an excellent film forming polythiophene organic semiconductor. Bottom contact organic field effect transistors (OFETs) were fabricated by spin-coating a 50nm film of the co-polymer atop the FET structure using a previously described procedure.

Figures 1a and b depict the electrical characteristics of the OFET having co-polymer 3 as the channel. Figure 1a shows the drain-source currents of the OFET as a function of drain-source voltage for several gate-source voltages ($V_{GS}=0$, 10 and 20V) before (black lines and inset) and after (gray lines) exposure to the alkylator methyl iodide (CH$_3$I, 9). Figure 1b shows the drain-source current as a function of gate-source voltage for drain-source voltage of $V_{DS}=10$V measured the same OFET having co-polymer 3 as the channel and under the same conditions.

As can be clearly seen in the figure, prior to exposure to the alkylating agent the device exhibits standard FET output characteristics with a slight indication of doping, probably due to the exposure to atmospheric oxygen. The post exposure curves show considerably higher currents and a resistor like response while the effect of the gate bias is lost. This is a clear indication for high level p-type doping associated with the exposure to methyl iodide.

Consistent with the results presented in Figure 2A, the transfer characteristics of the OFET prior to exposure show current modulation due to the gate-source voltage. The post exposure gray horizontal line shows almost no dependence on the gate voltage. At zero gate bias, $V_{GS}=0$, the exposure of the OFET to methyl iodide enhances the current by almost two orders of magnitude while at $V_{GS}=-20$V the factor is only 2-3. Assuming that the mobility is charge density independent and not affected by the doping one can use the above results to estimate the doping density according to Equation 1, where $C_{ox}$ is the gate capacitance [F/cm$^2$], $V_{GS}$ is the gate source bias [V], $q$ is the elementary charge and $\rho_{2D}$ is the dopant surface density.

\[ \text{Figure 1: Output (a) and transfer (b) characteristics measured on a FET having co-polymer 3 as the channel. The black lines and the inset show the device response prior to exposure while the gray (overlapping in a.) lines show the response after exposure to methyl iodide, 9.} \]
Assuming the doping is uniformly distributed across the ~50 nm film the doping density is \( \rho_{D} = 1.75 \times 10^{18} \text{ cm}^{-3} \).

\[
\rho_{2D} = 2 \cdot \frac{1}{q} C_{ox} V_{GS} = 2 \cdot \frac{1}{1.6 \times 10^{-19}} 35 \times 10^{-9} \cdot 20 = 8.75 \times 10^{12} \text{ cm}^{-2}
\]

The detection scheme of co-polymer 3 based OFETs involves the formation of a new covalent bond between the analyte (the alkylating agent) and the polymer in the channel. As this process is not reversible, all available reaction sites are bound to react. The number of reaction sites (pyridine rings, see Scheme 3) represents also the upper detection limit of the device. The total weight of the polymer embraced by the source and drain electrodes, \( M \), is \( 10^{-9} \text{ g} \). (assuming a film density of \( 1 \text{ g/cm}^3 \), Equation 2, where \( W \) is the width of the device, \( L \) is the length and \( h \) is the thickness of the polymer film.

\[
M = W \times L \times h \times \rho = 1 \times 2 \cdot 10^{-4} \times 5 \cdot 10^{-6} \cdot 1 = 10^{-9} \text{ g}
\]

Co-polymer 3 consists of 5% (\( 5 \times 10^{-11} \) g) monomer 1 which are equivalent to \( \approx 1.5 \times 10^{13} \) moles. Consequently, reacting the OFET with \( \approx 9 \times 10^{10} \) analyte molecules will bring the system to saturation of all the active sites of the polymer. The OFET of co-polymer 3 produced similar results when exposed to a variety of alkylating agents, such as chloromethoxy ethane, 10, 1-chloro-2-ethylsulfanyl ethane (a close analog of sulfur mustard gas), 11, and benzyl bromide, 12. In contrast, exposure of the OFET to vapors of non reactive materials that are incapable of forming covalent bonds with co-polymer 3, such as methanol, 13, acetone, 14, water, 15, and isopropanol, 16, resulted with the deterioration of the output and transfer characteristics, as can be seen in Figure 2. A similar behavior of deterioration of the field effect characteristics could also be observed in OFETs lacking the ability to chemically react with alkylating agents such as OFETs having region regular poly-3-hexyl thiophene, rr-poly-2, as the active channel.

The effect of alkylation on FETs having co-polymer 3 as the channel can be rationalized by following the effect of alkylation on the charge distribution in the polymer. Co-polymer 3 is composed of electron rich polythiophene skeleton with pendant pyridine groups. Upon reacting with an alkylating agent, the pyridine group is transformed into an electron deficient pyridinium group. These electron poor groups attract charge density from the polythiophene skeleton, thus partially doping it.

![Figure 2](image-url)

**Figure 2:** Output (a) and transfer (b) characteristics of OFET having co-polymer 3 as the channel. The black lines show the device response at ambient conditions, while the gray lines show the response after exposure to methanol, 13.

As indicated above, the OFET readily reacts with any alkylating agent, producing the same type of reaction and thus cannot provide any straightforward information regarding the identity of the reacting alkylator.
Nevertheless, the OFET can provide valuable information regarding the reactivity of the alkylator. Figure 3 depicts the drain-source current as a function of the exposure time to vapors of methyl iodide, 9, at a constant gate-source voltage of -10V. When methyl iodide vapors are introduced to the atmosphere surrounding the OFET (marked with an arrow) the drain-source current drops, indicating the process of absorption of the vapors by the polymer and associated changes in its morphology and packing. The drop in the drain-source current is expected in view of the similarity between this process and what is found for non-reactive substances with OFETs containing co-polymer 3 and OFETs consisting of poly-3-hexyl thiophene, \textbf{rr-poly-2}, with any of the substances. Subsequently, the source-drain current starts increasing with time indicating that methyl iodide, 9, starts reacting with the polymer.

![Figure 3: Time response of an OFET having co-polymer 3 as the channel. Methyl iodide, 9, is introduced at T=10min (arrow).]

2.2 Detecting alkylating agents using chemomechanical asymmetrized microcantilever systems

Alkylation of nucleophilic nitrogen derivatives is able to induce irreversible effects other than changes in conductivity. In the next stage we have explored to ability to harness chemo mechanical effects to report an alkylation reaction.

![Scheme 4: Asymmetrized Si microcantilevers: a: picture of the roughened surface of the beams. b: SEM image of the intact face. c: SEM image of the roughened face. The length of the white bar is 500 nm.]

For this purpose we have manufactured asymmetric silicon microcantilevers that are capable of interacting with alkylation agents on one of their faces while their other surfaces are indifferent to the presence of alkylators, Scheme 4. The microcantilever of Scheme 4 is selectively etched on one of its faces according to a previously published procedure. The asymmetrized microcantilever is then immersed in a solution of poly-4-vinylpyridine, 17, in DMF for 20 minutes then removed from solution and dried. The asymmetrized microcantilever was exposed to vapors of the analyte and its guest induced bending was studied in a previously described system.
Figure 4 depicts the time dependent bending curves of the same microcantilever first exposed to vapors of methanol, $\text{CH}_3\text{OH}$ (11), in a nitrogen atmosphere then to a pure nitrogen atmosphere, then to a nitrogen atmosphere containing vapors of methyl iodide, $\text{CH}_3\text{I}$ (9), then to a pure nitrogen atmosphere again.

![Figure 4: Time dependent guest induced bending of a poly-4-vinylpyridine coated Octensis microcantilever in the presence of methanol, $\text{CH}_3\text{OH}$ (11), then methyl iodide, $\text{CH}_3\text{I}$ (9). Arrow 1 denotes start of guest flow, arrows 2 and 2a denote end of guest flow.](image)

As can be clearly seen in Figure 4 the absorption of methanol, $\text{CH}_3\text{OH}$ (11), to the polymer coated microcantilever is fully reversible. In the presence of the guest the microcantilever bends while in the absence of the guest the polymer bound methanol molecules are desorbed and the beam returns to its original bending. In contrast, upon exposure of the same microcantilever to methyl iodide, $\text{CH}_3\text{I}$ (9), a reaction occurs between the polymer and the guest. This reaction links the guest to the polymer through a new and stable bond. This cannot be reverted at room temperature and the deflection is partially irreversible, probably due to the desorption of some fraction of unreacted guest molecules.

The magnitude of bending depends on the nature of the alkylating agent, on its concentration and on the exposure time. Efforts are currently directed towards the identification of the alkylator through different properties of the bendogrammes.

3. Conclusions:
Organic field effect transistors and microcantilevers were harnessed to the detection of alkylating agents. Reaction of gas phase alkylators with a nucleophilic organic semiconductor in an OFET structure yields dramatic and irreversible changes in the transistor properties while other innocent molecules inflict reversible changes to the OFET parameters. In a similar manner, gas phase alkylators that react with a nucleophilic polymer at the surface of a microcantilever induces its irreversible bending while for innocent materials, such as alcohols, the bending is reversible and depends on the momentary concentration of the guest in the atmosphere.

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