Degradation studies of transparent conductive electrodes on electroactive poly(vinylidene fluoride) for uric acid measurements

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Received 18 December 2009
Accepted for publication 17 August 2010
Published 19 October 2010
Online at stacks.iop.org/STAM/11/045006

Abstract
Biochemical analysis of physiological fluids using, for example, lab-on-a-chip devices requires accurate mixing of two or more fluids. This mixing can be assisted by acoustic microagitation using a piezoelectric material, such as the β-phase of poly(vinylidene fluoride) (β-PVDF). If the analysis is performed using optical absorption spectroscopy and β-PVDF is located in the optical path, the material and its conductive electrodes must be transparent. Moreover, if, to improve the transmission of the ultrasonic waves to the fluids, the piezoelectric transducer is placed inside the fluidic structures, its degradation must be assessed. In this paper, we report on the degradation properties of transparent conductive oxides, namely, indium tin oxide (ITO) and aluminum-doped zinc oxide, when they are used as electrodes for providing acoustic microagitation. The latter promotes mixing of chemicals involved in the measurement of uric acid concentration in physiological fluids. The results are compared with those for aluminum electrodes. We find that β-PVDF samples with ITO electrodes do not degrade either with or without acoustic microagitation.

Keywords: lab-on-a-chip, acoustic microagitation, β-PVDF, ITO, AZO

1. Introduction

Efficient micromixing systems have been developed in order to overcome the limitations of mixing flows in microfluidic devices, achieving a complete mixing within a short time. Several approaches were attempted. Some are based on microelectromechanical system (MEMS) devices, such as microvalves and micropumps [1]. However, they increase the cost of the system and the complexity of its control and are difficult to integrate in a single chip. Other approaches rely on mixing by diffusion, requiring long or complex channels [2]. Such channels are difficult to microfabricate and can result in long mixing times, particularly for small diffusion coefficients of the involved fluids [3].

A different approach uses acoustic waves, both to promote mixing [4] and to pump [5] fluids. The acoustic waves can be excited with an ultrasound transducer, which can be fabricated from an electroactive polymer, such as poly(vinylidene fluoride) in its β-phase, β-PVDF [6]. Through electrical actuation, acoustic microagitation can be obtained, which enhances the mixing and shortens the reaction time without using moving parts [7].

In addition to its appealing piezoelectric properties, another interesting feature of β-PVDF is its transparency. Indeed, if the analytical measurement method is based on
optical absorption spectroscopy, it requires that both $\beta$-PVDF and the corresponding conductive electrodes, deposited on the microfluidic structures, be transparent to visible light.

Acoustic microagitation based on the piezoelectric $\beta$-PVDF polymer was applied to mix a urine sample with the uric acid reagent [7], using a cuvette with the $\beta$-PVDF polymer glued on its outside wall. It was concluded that, with acoustic microagitation, the mixing time decreases to 71% of the mixing time without microagitation. However, when a lab-on-a-chip device is used for biochemical analysis of physiological fluids by optical absorption, owing to microfabrication constraints, the ultrasonic transducer should be located in the optical path. Moreover, the reaction rate is expected to improve upon placing the film inside the microcuvettes, because the ultrasonic waves will be transmitted more efficiently from the ultrasound transducer to the fluid in the absence of an interface between them.

Before the lab-on-a-chip device fabrication with the ultrasonic transducer, preliminary experiments were performed using high-performance liquid chromatography (HPLC) and regular cuvettes with $\beta$-PVDF. The $\beta$-PVDF and its electrodes were placed on the internal walls of the cuvettes and the electrical contacts were electrically isolated with a suitable resin. Different compounds were mixed in regular cuvettes, but not in the microcuvettes of the lab-on-a-chip device, owing to the amount of solution required for the degradation study by HPLC. It was considered that the size of the films does not affect the degradation process; that is, if a film does not degrade at the macroscale, it should also remain unaffected at the microscale.

The tested electrode materials were aluminum, indium tin oxide (ITO) and aluminum-doped zinc oxide (AZO). This procedure was used to verify whether ITO and AZO electrodes degrade when in contact with certain chemical reagents and to choose the electrodes that fit better the required application and further fabrication of a lab-on-a-chip device. Electrode degradation would cause sample contamination and loss of microagitation efficiency. The Al electrodes were tested to verify whether Al is responsible for the degradation of the AZO electrodes.

The first issue in the degradation studies is the analysis of the chemicals involved in the concentration measurements of uric acid in physiological fluids. The associated chemical reaction is shown in figure 1 [8].

Uric acid (2,6,8-trihydroxypurine) is the main end product of purine metabolism; therefore, its determination in physiological fluids is of great importance and interest in the diagnosis and therapy of diseases caused by disorders of purine biosynthesis and purine catabolism, such as gout, hyperuricemia and Lesch–Nyhan syndrome [9]. Allantoin is a natural end product of purine degradation in most mammals. In humans and primates, owing to the lack of uricase, the end product of this catabolic pathway is uric acid [10]. Thus, it is possible to determine, indirectly, the uric acid concentration by calculating the allantoin concentration.

However, the allantoin concentration is difficult to measure owing to the lack of characteristic UV absorption features at around 260 nm in purines [11]. The determination of allantoin in biofluids is based on the Rimini–Schryver reaction; allantoin is converted to its glyoxyllic acid and then derivatized with 2,4-dinitrophenylhydrazine to glyoxylate-2,4-dinitrophenylhydrazone before analysis [12]. Other methods of allantoin determination include colorimetry [13], reversed-phase HPLC [14] with a precolumn [15] and ion chromatography [16]. In this work, HPLC was used to measure the allantoin concentration in the mixture of a urine sample and the uric acid reagent and to evaluate the degradation of $\beta$-PVDF and its electrodes during the reaction.

2. Experimental procedures

2.1. Instrumentation

We used a model 875 high-performance liquid chromatograph (Jasco), equipped with an UV detector set at 226 nm. Measurements were performed at a room temperature of 23°C on a Phenomenex C18 reversed-phase column (250 × 4.60 I.D., 5 $\mu$m particle size) [14]. The mobile phase was 10 mM potassium dihydrogen phosphate solution (pH 4.7) and the flow rate was 1.2 ml min$^{-1}$.

2.2. Reagents

Allantoin, potassium dihydrogen phosphate and uric acid were purchased from Sigma (Steinheim, Germany), and the uric acid reagent was purchased from Far Diagnostic. All chemicals were used as received.

2.3. Standard solution

The stock standard solution (0.9 mM) of allantoin was freshly prepared in water. Working standards were prepared immediately before injection by adjusting the pH to 7 with 0.01 N sodium hydroxide or 0.01 N sulfuric acid. We used 20 $\mu$l for each injection. Before the injection, all solutions were introduced into an ultrasonic bath and filtered using a Millipore filter (0.22 $\mu$m pore size, 25 mm diameter).

Figure 1. Scheme of the chemical reaction between uric acid, oxygen and water [8].
that did not undergo degradation. The shows the chromatograms of the samples presents the chromatograms obtained for the shows the chromatograms of the degraded samples described in table 1 that did not undergo degradation. The chromatograms for reaction times of 10 and 20 min are similar; therefore, the conclusions will be the same as those presented here.

In Al30, Almix30 and AZOmix30 solutions, allantoin was obtained (the number indicates the time, in minutes, that the material was in contact with the solution). However, in the Al30 and Almix30 solutions, a new compound was formed. Even more compounds were detected in the AZOmix30 solution, indicating that degradation did occur and that this electrode is not adequate for acoustic microagitation inside the cuvette. The results indicate that Al is the component responsible for the electrode degradation, because it is present in all the samples that degraded. Moreover, the AZO electrodes degrade only when acoustic microagitation is applied, suggesting that the following factors may be responsible for the degradation. Agitation generates heat that can detach particles from the film; this detachment can be enhanced by the poor adhesion of the film to the polymer.

Figure 4 shows the chromatograms of the samples described in table 1 that did not undergo degradation. The chromatograms for reaction times of 10 and 20 min are
Table 2. Experimental results obtained for samples that did not degrade at different reaction times.

| Reference       | Reaction time (min) | Area (mV min) | Peak intensity (a.u.) | C (mM)       | C (mM) (±0.001) | T (min) |
|-----------------|---------------------|---------------|-----------------------|--------------|----------------|---------|
| White10         | 2.207               | 61.760        | 10.666                | 0.20502      | 0.205 ± 0.001  | 2.202   |
| White20         | 2.200               | 61.485        | 9.525                 | 0.20415      | 0.204 ± 0.001  | 2.202   |
| White30         | 2.200               | 62.114        | 10.710                | 0.20613      | 0.206 ± 0.001  | 2.202   |
| β−PVDF10        | 2.179               | 61.69         | 10.669                | 0.20480      | 0.204 ± 0.001  | 2.202   |
| β−PVDF20        | 2.181               | 61.54         | 9.711                 | 0.20432      | 0.204 ± 0.001  | 2.202   |
| β−PVDF30        | 2.182               | 61.85         | 10.556                | 0.20530      | 0.205 ± 0.001  | 2.202   |
| AZO10           | 2.170               | 61.165        | 12.258                | 0.20196      | 0.201 ± 0.001  | 2.202   |
| AZO20           | 2.167               | 60.789        | 12.396                | 0.20513      | 0.205 ± 0.001  | 2.202   |
| AZO30           | 2.167               | 61.795        | 12.653                | 0.20314      | 0.203 ± 0.001  | 2.202   |
| ITO10           | 2.170               | 62.825        | 12.317                | 0.20112      | 0.201 ± 0.001  | 2.202   |
| ITO20           | 2.173               | 60.523        | 12.213                | 0.20460      | 0.204 ± 0.001  | 2.202   |
| ITO30           | 2.170               | 61.627        | 12.089                | 0.20837      | 0.208 ± 0.001  | 2.202   |
| ITOmix10        | 2.173               | 71.489        | 15.552                | 0.23564      | 0.235 ± 0.001  | 2.202   |
| ITOmix20        | 2.173               | 71.15         | 14.567                | 0.23457      | 0.234 ± 0.001  | 2.202   |
| ITOmix30        | 2.173               | 71.405        | 14.905                | 0.23538      | 0.235 ± 0.001  | 2.202   |

Table 2 reveals that the retention time is approximately 2.2 min for all solutions, independent of the acoustic microagitation time; therefore, there is no reaction between the different electrodes and β−PVDF. After the first 10 min, the acoustic microagitation time does not affect the allantoin concentration. On the other hand, acoustic microagitation using ITO electrodes results in a higher allantoin concentration than the case without acoustic microagitation, proving that the microagitation promotes the reaction in our cuvette and that it can promote the reaction in the microcuvettes of a lab-on-a-chip device.

4. Conclusions

This work demonstrates that β−PVDF films with ITO electrodes do not degrade and do not negatively affect the uric acid analysis. Therefore, they can be deposited inside the microfluidic structures of a lab-on-a-chip device or similar devices to achieve an efficient acoustic microagitation, inhibiting the formation of an interface between the transducer and the fluid. When β−PVDF films containing aluminum were used as electrodes, even at low Al concentrations, such as that observed in the transparent conductive AZO, they degraded either with or without acoustic microagitation. We therefore conclude that aluminum is responsible for the degradation of AZO electrodes during the analysis of uric acid.

Acoustic microagitation using ITO electrodes results in a higher allantoin concentration than the case without acoustic microagitation, demonstrating that the microagitation promotes the associated chemical reactions.

In future studies, it would be useful to identify the degradation products of the HPLC measurements and to measure the concentration of allantoin generated from a real body fluid sample containing not only uric acid but also more complex compositions.
Acknowledgment

Support for this research was provided by the Portuguese Foundation for Science and Technology (grants PTDC/BIO/70017/2006, PTDC/CTM/69362/2006 and SFRH/BD/44289/2008).

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