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Chemically Polymerized Polypyrrole for On-Chip Concentration of Volatile Breath Metabolites

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Abstract

A wide range of metabolites are measured in the gas phase of exhaled human breath, and some of these biomarkers are frequently observed to be up- or down-regulated in certain disease states. Portable breath analysis systems have the potential for a wide range of applications in health diagnostics. However, this is currently limited by the lack of concentration mechanisms to enhance trace metabolites found in the breath to levels that can be adequately recorded using miniaturized gas-phase sensors. In this study we have created chip-based polymeric pre-concentration devices capable of absorbing and desorbing breath volatiles for subsequent chemical analysis. These devices appear to concentrate chemicals from both environmental air samples as well as directly from exhaled human breath, and these devices may have applications in lab-on-a-chip-based environmental and health monitoring systems.

Keywords

polypyrrole; breath analysis; pre-concentration; adsorbent; biosensor; MEMS; volatile organic compounds (VOCs); biomarker

Introduction

Conductive polymers are widely studied because of their electroactivity, stability and redox properties [1,2]. In particular, polypyrrole (PPy) and its derivatives are of great interest for several reasons. Pyrrole is easily polymerized via electrochemical or chemical methods, it is capable of electrochemical switching, and it is biocompatible. In addition, the pyrrole monomer is readily available commercially. Applications of PPy include microelectronics, optical sensors, batteries, and volatile organic compound (VOC) detection [3,4]. PPy films are advantageous for use in solid-phase microextraction (SPME) [5-8] due to their electroactivity and reversible redox properties. These films are largely electrodeposited onto wires, similar to those used in traditional SPME experiments, and these PPy SPME fibers have been used to

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concentrate both inorganic compounds [9] and chemicals from biological sources such as human plasma [10].

The electrochemical polymerization (electropolymerization) of pyrrole is well documented [1,11,12] and commonly performed due to its ease of fabrication. However, there are several disadvantages to electropolymerization. Primarily, surface morphology is difficult to control often yielding rough surfaces [8,13,14]. In addition, accurate thickness control above 100 μm is challenging and mass production is limited with this method [15]. The chemical polymerization of pyrrole is advantageous for producing films with large, uniform surfaces [16]. Chemically polymerized films can be applied to many different substrates and their surface dimensions and characteristics can be easily modified with a variety of methods such as patterning or particulate leaching. This sets the stage for using patterned PPy materials in microelectromechanical systems (MEMS) and nano-devices, especially those developed for lab-on-a-chip applications.

The analysis of volatile and non-volatile compounds in exhaled human breath provides a desirable and promising platform for the diagnosis of various diseases and disorders including cancer, asthma, respiratory infections and potentially many others [17-19]. Many methods and commercial or custom-made collection devices exist for the collection of exhaled human breath for subsequent chemical analysis of the samples [20,21]. One method is to collect filtered breath through the use of sampling bags [22,23]; another is to collect only the exhaled breath condensate (EBC) for sampling [24,25]; and a third method is to concentrate exhaled breath directly onto a substrate such as a carbon-activated sorbent [26,27] or solid-phase microextraction (SPME) filters [28,29]. Each of these methods has potential limitations.

In this work, we describe a new method of creating chemically-polymerized PPy films on-chip to function as a concentration device to capture exhaled breath metabolites. The cartoon in Figure 1 illustrates the function of this device. Briefly, a PPy film is exposed to a continuous stream of exhaled human breath (Figure 1A). Breath samples contain many rare and abundant compounds, including both volatile and non-volatile metabolites, and some of these metabolites are selectively absorbed on the PPy device, and become concentrated within the polymer matrix (Figure 1B). When the device is later heated, these concentrated breath metabolites are then desorbed and can be detected using a variety of gas-phase sensors (Figure 1C). By reducing the PPy concentration devices to chip-based miniature formats, we can later incorporate them into mobile breath analysis sensor systems for human health diagnostics.

Materials and Methods

Preparation of Polypyrrole Films

Chemical polymerization of polypyrrole material has been previously reported [16,30-32], however the specific details of most of these protocols are not well documented in the literature. Our formulation is a modification of the acid-promoted polymerization of pyrrole first reported by Su, *et al.* [33]. The chemical sources of our film material were: trichloroacetic acid (TCA; EMD Chemicals), 99% pure tetrahydrofuran (THF; Sigma Aldrich), and 99% pure pyrrole monomer (Py; ACROS Organics). To prepare our film solution for deposition, 5 g TCA was fully dissolved in 5 mL THF and mixed with 25 mL of Py. The resulting mixture did not require subsequent filtering or additional processing prior to use.

The solution is patterned onto a substrate using a method similar to a microfabrication photoresist in a normal class 10,000 clean room environment. A $3 \times 1 \text{ inch}^2$ SiO_2 substrate was placed on a Solitec spinner and vacuum pressure was applied to steady the substrate for film deposition. Approximately 1.5 mL of pyrrole solution was deposited onto the substrate to cover the surface. The substrate was spun at a spreading speed of 500 rpm for 10 sec and a

coating speed of 2,150 rpm for 30 sec. At this point, the substrates were covered with a thin film layer of the pyrrole material, and the devices were then placed in a vacuum oven at 100 °C for 24 hours to cure the material. While we used a quartz SiO₂ substrate in this report, it is also feasible to use a silicon substrate as the base and to incorporate pyrrole material into microelectromechanical systems (MEMS) and devices.

Film Characterization

We used Fourier transform infrared (FTIR) spectroscopy (Bruker TENSOR) to characterize the chemical content of the film after initiating the chemical polymerization process. A profilometer was used to analyze the thickness and roughness of the films prepared using the details above. This resulted in an average film thickness of $0.197 \pm 0.0218 \mu\text{m}$ (n=9) and average surface roughness of $40.7 \pm 9.92 \text{ \AA}$ (n=9). Scanning electron microscopy (SEM) images were taken to determine surface properties of the PPy film. Conductivity measurements were performed with a standard two-probe multimeter and a four-probe setup.

Exhaled Breath Sampling

We collected exhaled metabolic breath analytes using our patterned PPy thin film devices. Briefly, the PPy material was deposited as described above, and the substrate was diced into testing devices that were $25.4 \times 11.1 \text{ mm}$ in dimension for breath sampling. These diced devices were inserted into the end of a commercial RTube™ breath collection device (Respiratory Research, Inc., Charlottesville, VA), which we used to standardize collection and allow for focusing of the exhaled breath stream across the PPy devices. During breath sample collection, a -80 °C pre-chilled insulated aluminum cooling sleeve was placed around the exterior breathing tube to chill the PPy devices and to promote collection of both the exhaled breath volatile and non-volatile metabolites onto the chips. In this present report, we examine only the volatile fraction of the breath metabolomic signature that is concentrated by the PPy devices.

Breath analytes were collected onto the PPy devices for 1, 2 and 5 minute time points (n=3 replicates each). A control experiment was also performed for each time point by exposing a PPy film to ambient air. This allowed us to determine which chemicals were simply concentrated and absorbed from the ambient room environment where the human subject was located and which chemicals appeared to be analytes concentrated from the exhaled breath stream. After collection, the PPy devices were immediately placed into 10 mL headspace sampling borosilicate vials with Teflon septa and capped (Agilent). An empty blank vial with no PPy device was also analyzed to quantify the ambient baseline background.

These clinical samples were collected under an approved IRB protocol that allowed for recruitment of normal healthy controls to provide breath samples for method and instrument development of new technologies (IRB certification #2007151288-2, PIs: Kenyon and Davis, Study Title: "CCRC: Detection of Novel Biomarkers of Asthma and COPD in Exhaled Breath"). All trainees who worked on this effort were required to certify IRB- and data-handling compliance by completing an online course on human subject trials.

Chemical Analysis of Concentrated Volatiles

Gas analysis was performed using a Varian 4000 gas chromatography mass spectrometer (GC/MS). An 85 μm polyacrylate solid-phase microextraction (SPME) fiber (Supelco Inc., Bellefonte, PA) was used to extract compounds from the headspace above the PPy devices and inject the volatile analytes into the GC/MS for analysis. Briefly, the PPy devices which were contained inside of sealed borosilicate vials were heated to 100 °C for 20 minutes to equilibrate and release the captured breath chemicals into the headspace of the vial for subsequent chemical analysis. The SPME fibers then extracted chemicals from the headspace for 45 minutes prior

to injection of the fiber into the gas chromatograph where it was allowed to desorb for 7 min 50 sec. Between experiments the SPME fiber was heated and conditioned to remove residual volatiles for 90 min at 250 °C.

The chemical analysis was performed using a Varian 4000 GC/MS (Varian, Inc., Walnut Creek, CA) and used electron impact ionization. We used a CombiPAL autosampler and a 30 meter VF-5ms capillary column (Varian Inc., Walnut Creek, CA). When the samples were injected onto the head of the column, we used cryo-cooling by using liquid nitrogen to reduce the temperature of the GC oven to 5 °C, which increased the sharpness of chromatographic peaks for the analytes we measured. The GC column temperature was increased by 1.0 °C/min from 5 °C to 125 °C and by 5.0 °C/min from 125 °C to 200 °C. The carrier gas was 1 mL/min helium, the m/z range was 35-400, and the injection was splitless to boost the signals from low abundant molecules in the samples.

The GC/MS data was analyzed using the MS Workstation 6.6 software (Varian, Inc., Walnut Creek, CA). The total ion chromatographic peaks were recorded as the signal output, and the mass spectrum associated with each scan were viewed and compared to the NIST mass spectral library for compound identification.

Results and Discussion

Device Manufacturing and Characterization

Films of the polymerized polypyrrole (PPy) material were fabricated and deposited onto SiO₂ substrates using the techniques described in the methods section. To confirm that we had fully polymerized the pyrrole, we collected Fourier transform infrared spectra from the samples and this is presented with the prominent peaks labeled in Figure 3. The chemical formula of pyrrole is C₄H₅N, and PPy has the formula [C₄H₄N]_n (Figure 2). By examining The FTIR spectrum obtained for polypyrrole and comparing this to published spectra [15,33,34], the major peaks can be attributed to PPy structural features (Table 1). The peaks shown at wave number 3368 cm⁻¹ and 3098 cm⁻¹ represent stretching of the N-H and C-H bonds, respectively. The peaks at 1653 cm⁻¹ and 1560 cm⁻¹ represent a stretching of the carbon double bonds present in the polypyrrole structure, while the peak at 1252 cm⁻¹ corresponds to the C-N bond stretch present in amines [15]. The strong peak at 777 cm⁻¹ is likely due to a combination of C-H and N-H out-of-plane bending. Two peaks were present in the spectrum at 2932 cm⁻¹ and 2855 cm⁻¹, which likely correspond to the two absorbance bands expected from CH₂ bonds [33] of which there are 4 present in each solvent THF molecule (Figure 4). The existence of these bands in the spectroscopy data is not surprising and is likely residual THF that remains after polymerization of the PPy material.

Scanning electron micrograph (SEM) images were obtained from the PPy devices, and illustrate the extreme smoothness of the films (Figure 5). In order to obtain a clear image, surface deformities near the diced edge were used to focus the image. The apparent lack of surface features agrees with the profilometer measurements taken from the devices which was 40.7 ± 9.92 Å (n=9 samples).

Polypyrrole is of great interest in part due to its conductivity. Chemically polymerized pyrrole has reported electrical conductivity from 5 to 200 S/cm [4,35-37] whereas electropolymerized pyrrole range from 20-500 S/cm [1,38] and up to 1000 S/cm for elongated films [39]. Resistivity measurements of the prepared polypyrrole films and bulk polypyrrole were attempted with a multimeter and a four-point probe as described above. The tests for both materials returned infinite resistance implying little conductivity with the specific formulations in this report. There are several potential contributing factors to this phenomenon, including the choice of solvent and/or acid used in the formulation.

Machida et al. demonstrated the effect of solvent choice on conductivity, ranging from 0 S/cm for acetone and 20 S/cm for THF up to 120 S/cm for pentanol and over 100 S/cm for water [37]. The process in our present work is similar to that of Su et al. [33] except that trichloroacetic acid is used in place of perchloric acid. Perchloric acid has an acid dissociation constant (pK_a) of -7 which is considered a very strong acid, whereas trichloroacetic acid has a pK_a of 0.77 and is generally considered a weak acid. In addition, Su et al. proposed the existence of two polymer phases: an ordered and well-crystalline phase and a less ordered and less-crystalline phase. Linear structures were present in the well-crystalline phase and were hypothesized to help promote charge carriers along the chains, thus resulting in a measurable conductivity. In future formulations, it may be possible to tune the film conductivity for our desired breath analysis devices so that the films are conductive and can be patterned into resistive heating elements. This would allow us to use the PPy devices as both a breath analyte absorbing and concentrating element, and then to use the conductive nature of the film to heat the material and desorb the biomarkers on demand for on-chip gas analysis. This could be a tremendous advantage for portable breath diagnostic instruments that are designed for point-of-care clinical settings.

Device Use for Breath Metabolite Characterization

We opted to utilize a commercial breathing apparatus to focus and stream exhaled breath over our PPy concentration devices to test for metabolite absorption and capture by the polymer film. The polypropylene commercial R-Tube™ device consists of a mouthpiece connected between two one-way valves with an exhaled breath condensate (EBC) collection tube surrounding and extending from the exhale valve. Typically these devices are used with an aluminum cooling sleeve surrounding the breathing tube which condenses the exhaled breath as it passes through. During normal use, EBC condenses along the interior walls of the tube, where it can be collected and harvested for subsequent chemical analysis. In this study, we inserted our customized PPy concentration devices into the proximal end of the R-Tube™ to absorb volatile breath metabolites directly from the stream of breath being exhaled from human subjects. The polypyrrole concentration devices were used as an extraction phase to capture and concentrate human breath volatiles for chemical analysis. The PPy chips were then analyzed using GC/MS as described in the methods section after applying heat to desorb the concentrated metabolites that collected onto the film.

When the resulting chromatograms from the PPy devices were analyzed, we found various chemicals could be concentrated onto the films (Figure 6A) and there appeared to be three distinct categories of chemicals that were concentrated by the PPy devices. First, we found that various chemicals from the ambient room environment air could be concentrated and then measured from the PPy devices (Figure 6B). This suggests that the devices are functioning as intended and are capable of absorbing and desorbing volatile chemicals that they encounter. Secondly, we found that some chemicals that we desorbed from the films were found only in devices that were exposed to human breath, and were not found in the recordings taken from devices exposed to ambient air environments (Figure 6C). This indicates that some fraction of the compounds desorbed by the devices are likely to be due to breath metabolites, and that this method of collection is capable of capturing and concentrating these compounds for subsequent chemical analysis. And finally, we found that compounds from the PPy device itself can be measured in the resulting GC/MS spectra, such as the case of tetrahydrofuran (THF) which is the solvent used in the device manufacturing method (Figure 6D). In this last case, we found that THF was observed as a peak in the chromatograms from both the environmental air sample as well as the exhaled human breath sample, as indicated (*, Figure 6D), but not in the blank background control. Interestingly, we also found what appeared to be a furan-like related compound (**, Figure 6D) at a slightly earlier retention time that was found only in the chromatogram from the device exposed to human breath. This chemical may be due to residual

THF that was released from the device combining with an actual breath metabolite that was concentrated onto the device, which would account for the absence of the molecule in the control and ambient air device samples.

The PPy devices appeared to concentrate a fraction of volatile compounds that are present in exhaled breath samples from normal healthy individuals (Figure 7). The chromatograms that resulted from desorbing the chemicals from the PPy films were examined to determine if there were peaks present in the human exposed devices that were not present in the ambient environmental or blank controls. In our preliminary study, at least 16 spectral peaks were discovered, which were different from controls, and these were recorded across a variety of retention times. Nine additional chemical peaks were also identified as distinct from the control data; however, careful inspection of these signals showed that the peaks were either of too low amplitude to accurately quantify, or the peaks could have resulted from time-misalignment in the spectral signal, which is common in chromatography [40]. These additional peaks are not shown here, however they indicate that additional future work may find that a wider array of analytes can be absorbed onto the PPy chips than even those presented here.

We attempted to determine the chemical identity of these putative breath metabolites by comparing the mass-to-charge ratio from the spectra to the National Institute of Standards and Technology (NIST) library database (Figure 8). Several of the prominent breath metabolites were matched to NIST library targets (Table 2); however, some chemicals could not be identified and did not appear in the database. The NIST library itself contains a relatively limited number of spectra references (under 200,000) and many are user-submitted data, again complicating exact chemical matching methods. In several of these cases, the compounds appeared related to discrete biological processes that are related to metabolism. For example, pregnane is a steroidlike derivative [41], allocholate is related to many bile acid products [42], panthetheine is a breakdown product of coenzyme A that is important in many metabolic processes [43], octadecatrienoic acid is another lipid-type compound related to metabolism [44], glucopyranoside is related to many lipophilic surfactants and flavonoids found in many foods [45], and finally spirost-derivative compounds are also found in many natural or unprocessed foods [46]. It seems likely that these putative breath compounds could arise from endogenous sources within the human body, and it is reasonable to suggest that we might detect them in human effluent. Several compounds were present at abundance levels that were too low to allow for accurate chemical identification and matching with the NIST library (denoted *, Table 2). We found that a reasonable number of breath analytes appear to be captured and concentrated by the PPy device, but the exact chemical identification of these will need to be attempted in future studies. It is also possible that extending sampling times or optimizing other operating conditions could increase the abundance of these and other concentrated biomarkers, and that PPy film modifications could expand the concentration range and capabilities of this device.

A variety of techniques could be employed in the future to modify the PPy surface characteristics, analyte sensitivity and selectivity. One method would be to incorporate counter ions into the film during polymerization. Wu et al. [13] report the incorporation of different counter ions into a PPy film, and depending on the size and nature of the counter ions, the resulting film is vastly different. Small anions incorporated into the PPy film increase the anion exchange property of the film, whereas larger ions such as poly[styrenesulfonate] (PSS) increase the cation exchange property. In addition, films incorporated with large anions were the most mechanically and thermally stable. Sadki et al. [1] demonstrated an increase in conductivity with the incorporation of large cations and Sahin et al. [14] reported that the doping of conducting organic polymers could be useful for pre-concentration and separation of analytes. These reported findings suggest that the conductance and ion exchange properties

of PPy may be linked, and represent potential approaches to fine-tune the breath analyte concentration capabilities of our device.

Further research on PPy fabrication may also produce more conductive films, so that the material could be patterned for dual-use as both a concentrator and a resistive heating element. This would allow a simple MEMS device to be created that could both absorb and concentrate volatile breath metabolites when operating in a passive regime, and then function as a resistive heating element to desorb the compounds when triggered for subsequent on-chip chemical analysis. Two potential routes to increase the film conductance would be to reduce the polymerization time and/or temperature [47] or to modify the constituent ratios used in the reaction. The solvent could also be changed to one with higher reported conductivity, such as methanol or water [37]. Another option is to substitute the acid with one such as perchloric acid that is reported to achieve conductive films [33]. Finally, an additional method to achieve conducting films is with the introduction of a dopant. A dopant will not only increase the conductivity of polypyrrole [36,38], but it may also increase the concentration and separation of anionic [5,48], cationic [9,48,49] and neutral analytes [50,51].

Conclusions

With any medical diagnostic instrument, a short sampling time is preferred. This can be achieved with breath analysis by concentrating low abundant trace volatile breath metabolites so that they can be more easily monitored. This report characterizes the VOC capture efficiency of exhaled breath onto chemically polymerized polypyrrole (PPy) chips. The PPy films were prepared via acid-promoted chemical polymerization on a glass substrate to achieve a large surface area and smooth surface for the adsorption and concentration of volatile breath biomarkers. The pyrrole monomer was polymerized using trichloroacetic acid as the reaction catalyst and took place in a solution of tetrahydrofuran to provide a steady reaction. FTIR data demonstrates the presence of polypyrrole in the final devices, and SEM images demonstrate a smooth film is generated using this method.

Ultimately, we believe that patterned PPy films can be a critical component in a miniature breath analysis system. The development of micro total analysis systems (μ TAS) is an active field, one that has grown considerably in the past few years [52]. Miniaturization is especially important for the real-time analysis of gaseous species, and this includes diagnostic breath tests. In addition to increased portability, miniaturization of this class of devices may also increase sensitivity of overall detection, due to the concentration effect on trace metabolites. A decrease in power consumption in the mobile systems and faster analysis times [17] could contribute to entirely new generations of mobile breath analysis platforms.

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Author Biographies

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Figure 1. Cartoon illustrating the function of a polypyrrole (PPy) patterned on-chip concentration device. As a subject exhales (A), volatile organic compounds (VOCs) breath metabolites pass over the PPy concentration device. As these molecules are absorbed (B), they accumulate onto the PPy film over several minutes of exposure. The device is later heated to desorb and release the breath metabolites as a concentrated “plug” of material for subsequent chemical analysis (C).

Figure 2.
Chemical structures of pyrrole (Py) and polypyrrole (PPy) in panels A and B, respectively.

Figure 3. Fourier transform infrared spectroscopy (FTIR) data for the PPy thin film device. Prominent spectral peaks are highlighted for further analysis, and these are reported in Table 1.

A **B**

Figure 4. Chemical structures of the solvent component (A) tetrahydrofuran (THF) and the polymerizing agent (B) trichloroacetic acid (TCA) used to create the PPy devices.

Figure 5. Scanning electron micrograph (SEM) shows surface characteristics of an example PPy device have extremely low roughness and are very smooth.

Figure 6.

Example total ion chromatogram (TIC) showing that various chemicals were concentrated onto the PPy device (A). There appeared to be three categories of compounds that were resolved, and examples peaks are shown for these groups: (B) ambient chemicals concentrated from the sampling environment; (C) exhaled breath metabolites that were collected during human sampling; (D) chemicals that were related to the tetrahydrofuran (THF) solvent chemical that was used to form the PPy device itself. In this last case, the THF chemical peak is marked (*) and is seen in the blank and environmental and breath samples. There was also a closely related furan-type chemical observed in the human breath sample (**) that was measured in addition

to the THF, and this may be an actual breath metabolite complexed with a derivative of the THF solvent from the PPy device.

Figure 7.

At least 16 compounds desorbed from the PPy devices appeared to be concentrated metabolites from exhaled human breath. Chromatograms for each peak are shown and marked with a number at the highest point, and peaks that were concentrated from the environment are also noted (*).

Table 1

Analysis of FTIR data from PPy concentration devices. We use the major peaks to determine bond characteristics of the PPy thin film material.

Range (cm ⁻¹)	Actual ω (cm ⁻¹)	Forms of Vibration
3300-3400	3368	N-H stretch (secondary amines)
3020-3100	3098	C-H stretch
1630-1680	1653	C=C stretch
1510-1540	1560	C=C stretch
1450	1425	Aromatic C=C stretch
1260	1252	C-N stretch
1100	1092	C-C-C bending
1060-1070	1092	C-H in-plane bending
1040-1050	1025	C-H out-of-plane bending
870-890	883	C=C stretch
770-780	777	C-H out-of-plane bending

Table 2

Putative breath metabolites collected by the PPy device differed from the controls, and the baseline-subtracted amplitudes of the peaks in Figure 7 are reported here. Chemical matches were provided by the NIST Mass Spectral Library. Inconclusive matches are also noted (*), and one compound is possibly linked to the solvent THF which is used to form the PPy devices (**).

Peak #	RT (min)	Norm Peak Amplitude (counts)	NIST Chemical ID match
1	29.48	4,441	pregnane
2	55.59	3,969	ethyl iso-allocholate
3	56.05	16,817	*
4	59.71	15,141	*
5	60.11	16,822	*
6	91.74	7,504	2-myristynoyl panthetheine
7	100.44	2,851	octadecatrienoic acid
8	101.73	5,201	glucopyranoside
9	102.11	2,561	*
10	102.93	2,162	*
11	125.49	6,889	spirost-8-en-11-one
12	126.22	10,239	*
13	138.77	15,200	*
14	140.39	9,904	tetrahydropyran derivative (**)
15	146.64	9,893	*
16	147.23	14,149	*