This paper reports a novel method to detect ammonia by using the ninhydrin–PDMS composite. The polymer composite film is prepared by integrating ninhydrin into the PDMS polymer matrix. Further, an optical lab-on-a-chip device is developed by integrating the ninhydrin-polymer composite into a microfluidic device for the detection of ammonia. The chemisorption of ammonia onto the composite resulted in the change in its optical absorption property. The proposed device has an integrated light emitting diode and photosistor in order to measure the change in absorption and hence the detection and quantification of ammonia are performed. The response time of the sensor was found to be linear for a wide range of ammonia concentrations and it is shortest for the thin (100 μm) composite film. The limit of detection of the proposed device is found to be as low as 2 ppm. The proposed sensor platform is also demonstrated for the detection of amino acids.

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Micro devices have been extensively used for biological and chemical sensing applications due to their high sensitivity, use of minute amounts of samples and reagents, low cost, portability, etc. Presently, Polydimethylsiloxane (PDMS) is the most commonly used material for the fabrication of microfluidics devices and lab-on-a-chips. PDMS is an important structural material for the fabrication of micro- and nanodevices for various biomedical and industrial applications, because of its advantages including low-cost, biocompatibility, excellent optical properties, and simple and low-cost fabrication processes. Several attempts have been reported in literatures for enhancing the properties of PDMS. In order to realize a plasmonic sensor, the gold (or silver) - nanostructures are embedded into the PDMS matrix and, hence various microfluidic biosensors are realized. The reinforcing of PDMS with gold or silver nanoparticles and/or carbon nanotubes is demonstrated for enhancing the mechanical properties and adding the electrical conductivities for various electro-mechanical applications.

In the present work, we propose a novel method of using PDMS as sensing material for detecting ammonia and amino acids and for any N\textsubscript{2}H\textsubscript{4} groups, by integrating the ninhydrin reagent into the PDMS matrix, so that PDMS can be used for the realization of a variety of applications, including biosensing and indirect sensing application. In the present work, the feasibility of synthesizing the ninhydrin-PDMS nanocomposite and developing micro devices for detecting ammonia and amino acids are demonstrated. Ammonia is present almost everywhere in atmosphere at a low concentration, in the range of sub-ppb levels. Ammonia is a compound present in environmental samples, aquatic environments, in industrial wastewaters, plants and soil, in pharmaceutical formulations, etc. Most of the ammonia present in atmosphere is from human activities, among which agricultural activities such as livestock, fertilizers are the most prominent sources of ammonia. Other sources of ammonia include on-road vehicles, industrial chemical plants and silver chemicals. Extensive exposure to ammonia is harmful to animal and humankind. Ammonia at a level of 24–50 ppm can irritate the nose and the throat, but a moderate and higher level, typically above few hundreds of ppm of exposure to only a few minutes can cause severe irritation in the respiratory tract, spasms, and rapid suffocation. Acceptable threshold limit value (TLV) for ammonia gas with an exposure longer than 8 hours is around 25 ppm, and short-term exposure is 35 ppm for 15 minutes in order to avoid serious health issues. Hence, it is important to be able to detect ammonia in air, at low concentrations (ppm level) for controlling pollution and in industrial processes such as food technology, fertilizers, and, especially, in environments where refrigeration processes are carried out. Several methods have been reported for the determination of ammonia, including spectrophotometry, solid-phase extraction, diffuse reflectance spectroscopy, electrochemical methods, ion-chromatography, spectrofluorimetry, and capillary electrophoresis. Using the hypochlorite lumino chemiluminescence reaction, potentiometry with a differential system or indirect methods, for example, by amperometric detection of ammonium ions. Usually, the spectrophotometric method is used for the detection of ammonia and it is based on the adaptation of classical methods such as the Nessler, or the Berthelot reactions. The major application areas of ammonia sensors are gas sensing and analysis in automotive industry, chemical industry and biomedical industry. The ammonia sensors having high sensitivity can be used as disease diagnostic tool as the ammonia is natural body product and its detection and measurement can precisely predict the health conditions of various internal organs such a kidney and liver. It is well proven that the breath ammonia detection can be used as fast diagnostic technique for patients having kidney disorder or stomach ulcer.

There are many types of ammonia sensors that have been previously reported. They can be categorized into five main types and each of them have their own advantages and disadvantages. They are metal oxide semiconductor, catalytic ammonia sensor, conducting polymer gas sensors, optical gas sensing, indirect gas analyzers etc. Many of the classical analytical techniques used for the detection of ammonia suffer from drawbacks and are not suitable for the miniaturization. At present, there is an increased demand for miniaturized ammonia sensors due to several benefits such as faster analysis, low cost, easy to integrate with instruments or miniaturized air vehicles in order to map the presence of ammonia, for environmental or industrial safety monitoring etc.

As discussed, several sensing principles for measuring ammonia in air have been reported, however most systems such as infrared gas analyzers are too large and expensive, and not suitable for miniaturization and integration in a chip. Methods, more suitable for miniaturization such as those based on the semiconductive properties of metallic thin films (tin oxide or molybdenum oxides), or conducting polymer film gas sensors have also been reported. However, their detection limits are not low enough and the selectivity of the methods is poor for...
many applications. In addition, the sensors lifetime has been found limited. Other methods are based on the absorption of ammonia into a liquid and the subsequent detection of the ammonium ions by using an electrolyte conductivity detector.

Traditionally, the detection of gaseous ammonia was performed by electrochemical methods, which are sensitive and selective. However, the instruments are quite expensive and the presence of an experienced operator is necessary. Another kind of ammonia sensing devices, which were used for routine operations, are the commercial infrared gas analyzers. The infrared devices, although sensitive, are nevertheless expensive and bulky. Relative simple detectors, based on the semiconductive properties of SnO₂ and MnO₃ thin films can detect gaseous ammonia, but these devices have some restrictions concerning the reproducibility, stability, sensitivity, selectivity, and a limited active sensor lifetime. In recent years, several new approaches have been reported for the fabrication of optical gaseous ammonia sensors. These devices utilize the reaction of ammonia vapor with, either a pH-dependent dye material or a pH-sensitive film which undergoes a suitable color change or an absorption change. In general, these sensing mechanisms are based on monitoring the absorption or fluorescence characteristics of indicator dyes/sensing films entrapped within a membrane, deposited onto a wave guiding substrate or an optical fiber as substituted cladding. The targeted ammonia molecules interact with the immobilized indicator, resulting in changes in their absorbance or emission spectra, which are monitored using a proper detector module, via an optical fiber or planar waveguide. In the present paper, a novel ninhydrin-PDMS composite, having enhanced sensitivity for detecting ammonia is synthesized. The proposed method is suitable for integrating ammonia sensors in PDMS-based microdevices using the widely employed soft lithography technique.

Optical Detection of Ammonia by Using a Ninhydrin-PDMS Lab-on-a-Chip

Structure, general properties of ninhydrin and applications.—The structure of ninhydrin is shown in Figure 1. The reaction mechanism of ammonia with ninhydrine is given in Figure 2. The detection is based on the reaction of ninhydrine with ammonia (ammonium ion) as shown in Figure 2. The product of the reaction is a Schiff base, called Ruhemann’s Purple and the reaction is irreversible.

Principle of the method.—The detection is based on the reaction of ninhydrine with ammonia (ammonium ion) as shown in Figure 2. The product of the reaction is a Schiff base, a purple-colored and compound is very stable.

Figure 1. Chemical structure of ninhydrin.

Figure 2. Reaction of ninhydrin with ammonia.
Figure 3 shows the schematic of the ammonia sensor by using the Ninhydrin-PDMS composite. A PDMS platform containing the slot of 4mm wide channel for fixing the Ninhydrin-PDMS across an optical path is designed and fabricated. Two fluidic paths having width of 4mm are designed in order to guide the ammonium gas to the sensing element (Ninhydrin-PDMS composite film) as shown in Figure 3. The device is powered by 3V DC power supply. An LED (with emission wavelengths between 465nm and 475nm) and photo resistor were used to in the optical setup. The circuit diagram used for the biasing of LED and measurement of photo resistance value is shown in Figure 3. The voltage across the photoresistor is measured to quantify the amount of ammonia reacting with film. The optical setup measures the absorbance of PDMS-ninhydrin film when it reacts with ammonia and quantifies the concentration of ammonia.

Fabrication of the Ninhydrin-PDMS composite film.—As mentioned, the optical property of the ninhydrin changes when they react with the ammonia. However, in literature, the experiments have been carried out in the liquid phase of ammonia, using a sensor platform that measures ammonia only in liquid phase and is not useful for applications requiring a rugged and stable/portable sensing platform. Therefore, we are proposing a novel sensing platform of a polymer thin film containing ultrafine particles of ninhydrin. Herein, the polymer chosen is PDMS. PDMS has several advantages such as biocompatibility, good transparency in visible and UV light, easy to mold to any 3D complex geometry to fabricate microfluidics devices, and also the fabrication and material cost is lower.

A novel technology to fabricate the device was developed in-house and sensing films of 100–300μm thickness were fabricated. The synthesis of the ninhydrin-PDMS composite starts by mixing PDMS and the curing agent (10:1 wt%). Ninhydrin was dissolved in ethanol (0.5g in 5ml) and stirred until all the ninhydrin was dissolved. The ninhydrin solution was added to the PDMS mixture and stirred for about 5 minutes. Immediately after adding the ninhydrin solution, the PDMS mixture appears to have a low viscosity, then slowly, the PDMS mixture becomes viscous. Then, the mixture was degasified. The film was fabricated by spinning at 500 rpm for 30s, the PDMS-ninhydrin mixture on silicon wafer. The thickness of the film was between 100μm to 1mm. The film was baked at 85°C for 2 hours and peeled off from the wafer.

When the ninhydrin-PDMS composite film is exposed to the ammonium hydroxide solution, then color of the film changed from yellow to red pink as shown in Fig. 4a. The optical absorbance spectrum was also measured as shown in Fig. 4b. The absorbance spectrum of the composite film shows a high absorbance window between 420 and 480nm. Hence an LED with an emission band of 465–475nm was used in the device.

Fabrication assembly of the device.—Figure 5 illustrates the fabrication assembly process of the device. The PDMS platform containing a channel and slots for guiding the gas was bonded on a glass substrate using oxygen plasma bonding. The plastic horn shaped tubes, which were used as the guiding assembly for the gas to the sensing film, were fixed in the slots. The LED and photo resistors were integrated into PDMS. The sensing film was cut into 3 × 6mm pieces and affixed on a flat PDMS substrate Figure 5b and this substrate was used as the top lid of the PDMS platform as shown in Figure 5a. Figure 5c shows the sensor response has two well defined regions as shown in Figure 7, the transient region and the saturation region. This indicates that the sensor film slowly reacts with the ninhydrin and eventually gets saturated. It is noticed that the transient and saturation responses are strongly related with the concentration of ammonia and hence, both the responses can be used for quantifying the concentration of ammonia. When the concentration of ammonia was 15ppm, the transient response elapsed for around 100 seconds and then the sensor was saturated and the sensor output was stable. In these experiments, the thickness of the ninhydrin-PDMS composite film was 250μm. The saturated sensor output plotted against various concentrations of ammonia shows that, the sensor response is linear as shown in Figure 8.

Sensitivity test of the device.—Figure 7 shows the response of the device for various concentrations of the ammonium hydroxide solution. The concentration of ammonium was estimated and given in Figure 7. When the solution was passed through the channel, the ninhydrin-PDMS composite film turned to purple. The time response shows the response time of the sensor. The graph shows that the sensor response has two well defined regions as shown in Figure 7, the transient region and the saturation region. This indicates that the sensor film slowly reacts with the ninhydrin and eventually gets saturated. It is noticed that the transient and saturation responses are strongly related with the concentration of ammonia and hence, both the responses can be used for quantifying the concentration of ammonia. When the concentration of ammonia was 15ppm, the transient response elapsed for around 100 seconds and then the sensor was saturated and the sensor output was stable. In these experiments, the thickness of the ninhydrin-PDMS composite film was 250μm. The saturated sensor output plotted against various concentrations of ammonia shows that, the sensor response is linear as shown in Figure 8.

Study of sensor response time and thickness of composite film.—Further, we have carried out tests with various thickness of the film, and found that the response time can be reduced to a lower value, by reducing the thickness of the film. The limit of detection in the present case is found to be as low as 2ppm. Thecomposite was spun on a
Figure 5. The fabrication assembly of the device (a) PDMS platform with channel for gas and fluidic tube for guiding gas, LED and photoresistor (b) Top lid of the device with ninhydrin-PDMS composite (c) after bonding the top lid with PDMS platform.

silicon wafer to obtain various thicknesses and the response time was investigated. Figure 9 shows the response time with ninhydrin-PDMS film thickness of 100μm, 200μm and 300μm. The sensor response shows that a thin film’s response was the fastest (18 sec). It can be observed from the Figure 7 that the absorbance of the ninhydrin-PDMS film is slowly increasing linearly and after a few tens of seconds the absorbance became saturated. The slope of the linear response in the transient stage and the level of saturation of the sensor output were found to be a function of the concentration. The effect of thickness of the film and the concentration of ammonia is also investigated as shown in Figure 9, which shows that the slope of the transient response curve is independent of thickness of the film but the level of saturation only. From the experiments, it is proved that the sensor’s response time is 20 to 100 seconds; however, by using the slope of the response, we can estimate the concentration of ammonia within a very short time (less than 5–10 second), by measuring its slope as the film reacts with ammonia, as shown in Figure 10.

Detection of Amino Acids with Ninhydrin-PDMS

The feasibility of detecting amino acids with the developed ninhydrin-PDMS composite is also investigated by using glycine. In order to detect glycine, the absorbance spectrum of the ninhydrin-PDMS composite was measured when it reacts with glycine. The

Figure 6. (a) Device during testing (b) Device after packaging.
reaction of the glycine with the ninhydrin-PDMS composite was very slow at the room temperature and hence the composite was annealed at 100°C for 5 minutes. The heating process resulted in a faster reaction of composite with the glycine and the color of the composite changed to slightly blue. The absorbance spectrum of the composite film was also measured. Figure 11 shows the absorbance spectrum of the ninhydrin-PDMS composite after the reaction with the glycine. The spectrum has an absorbance peak at 570nm.

The sensor packaged with electronic hardware is shown in Figure 12. The sensor packaging includes a slot to insert the sensing film cut in the form of strips as shown in Figure 12 (Sensing film). After the film is exposed to the ammonia, the film will be inserted to the specially designed slot of the device in order to assess the optical absorbance characteristics of film at the specific wavelength (450nm), to perform the detection and quantification of ammonia. The sensor is interfaced with a microcontroller programmed to acquire data and analyses in the computer. Figure 13 shows the sensor response with the ninhydrin-PDMS composite film, before and after reacting with 30 ppm of ammonia.
USB interface to computer

Figure 12. Ammonia sensor with electronics hardware to interface with computer.

Figure 13. Sensor data with film before and after reacting with ammonia.

Conclusions

A micro optical based ammonia sensing platform is reported in this paper. The sensing platform is developed by synthesizing a novel PDMS-ninhydrin composite sensitive to ammonia. Further, the PDMS-ninhydrin composite is integrated in an optical microfluidic device and demonstrated for the detection of ammonia traces. The present device, can detect ammonia at a concentration as low as 2ppm. The platform is also demonstrated for the detection of amino acids present device, can detect ammonia at a concentration as low as 2ppm.

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