US 20120237968A1

(19) **United States**

(12) **Patent Application Publication**

Rigas

(10) **Pub. No.: US 2012/0237968 A1**

(43) **Pub. Date: Sep. 20, 2012**

(54) **DETECTOR AND METHOD FOR DETECTION OF H. PYLORI**

(76) Inventor: **Anastasia Rigas**, Setauket, NY (US)

(21) Appl. No.: **13/420,384**

(22) Filed: **Mar. 14, 2012**

Related U.S. Application Data

(60) Provisional application No. 61/452,391, filed on Mar. 14, 2011, provisional application No. 61/452,507, filed on Mar. 14, 2011.

Publication Classification

(51) **Int. Cl.**

C12Q 1/04 (2006.01)

C12M 1/34 (2006.01)

(52) **U.S. Cl.** **435/34**; 435/287.1

(57) **ABSTRACT**

An inexpensive, portable, hand held, point-of-care, non-invasive breath-analyzer for the detection of *H. Pylori* in adults and children by measuring ammonia in breath using a polyaniline-carbon nanotube sensor.

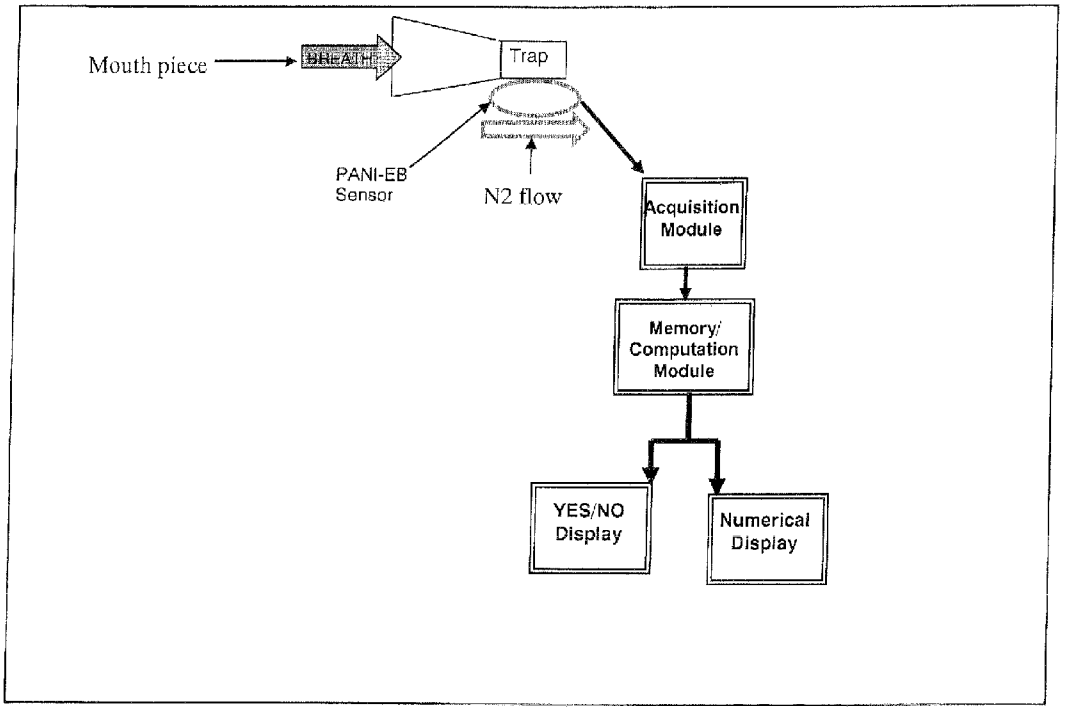
Mouth piece
BREATH
Trap
PANI-EB
Sensor
N2 flow
Acquisition
Module
Memory/
Computation
Module
YES/NO
Display
Numerical
Display

Figure 1

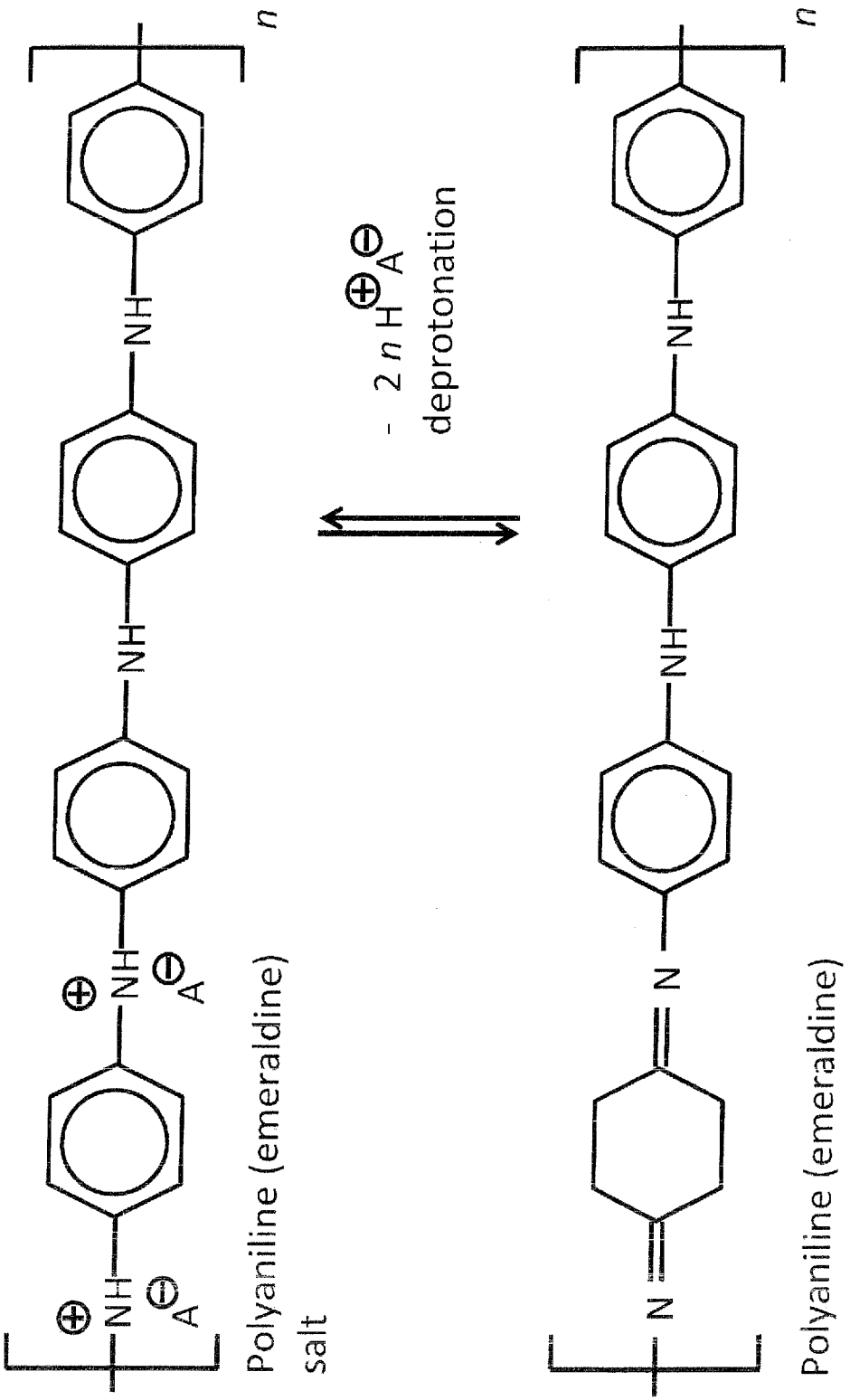
n
NH
NH
NH
A⊖
A⊖
Polyaniline (emeraldine) salt
- 2 n H⊕A⊖
deprotonation
n
NH
NH
N
N
Polyaniline (emeraldine) base

Figure 2

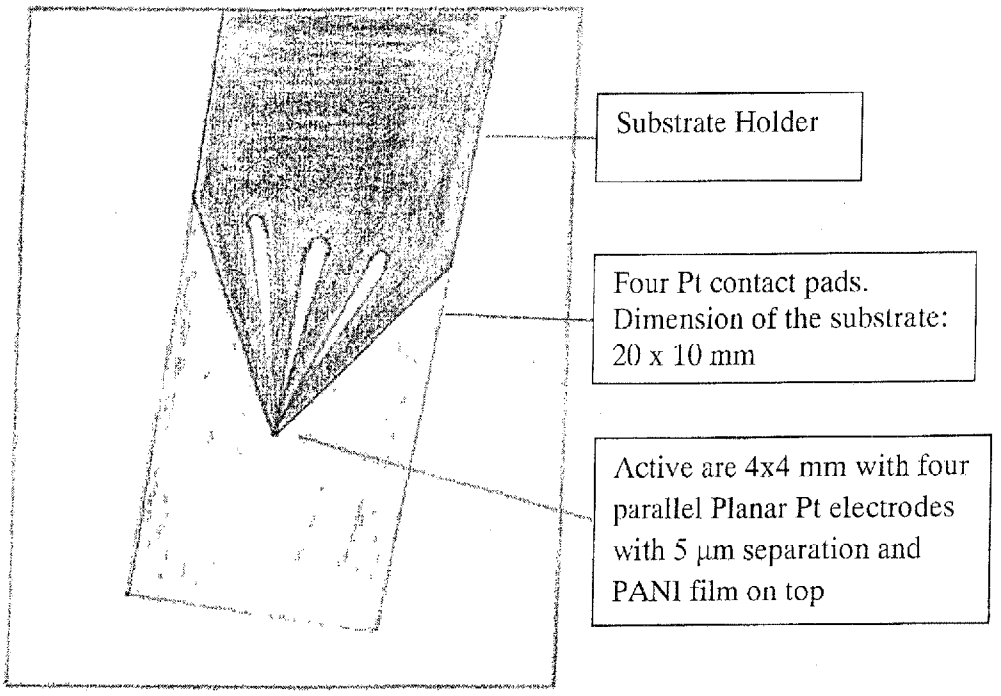
Substrate Holder
Four Pt contact pads. Dimension of the substrate: 20 x 10 mm
Active are 4x4 mm with four parallel Planar Pt electrodes with 5 μm separation and PANI film on top

Figure 3

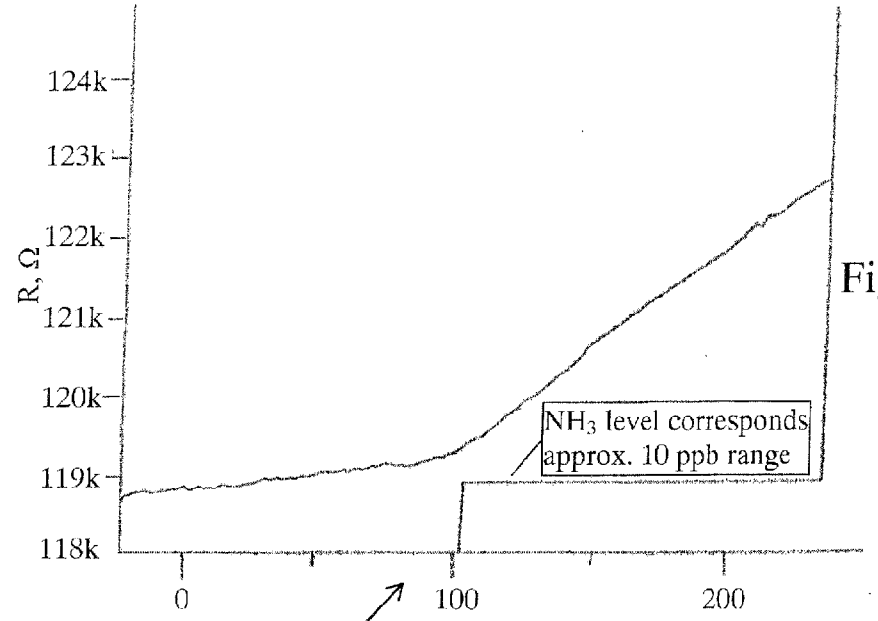

Figure 4(a)

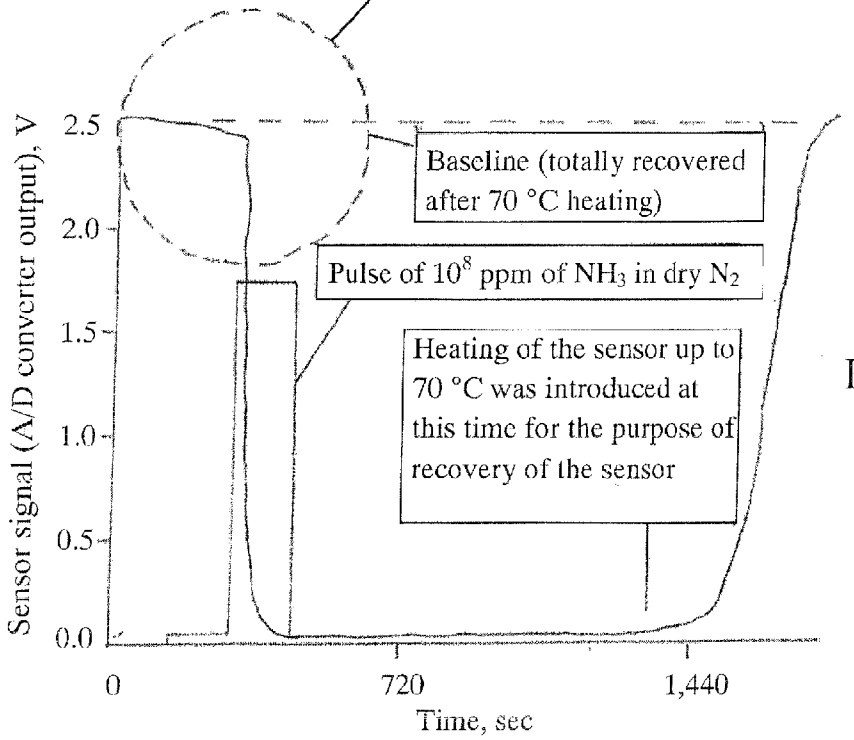

Figure 4(b)

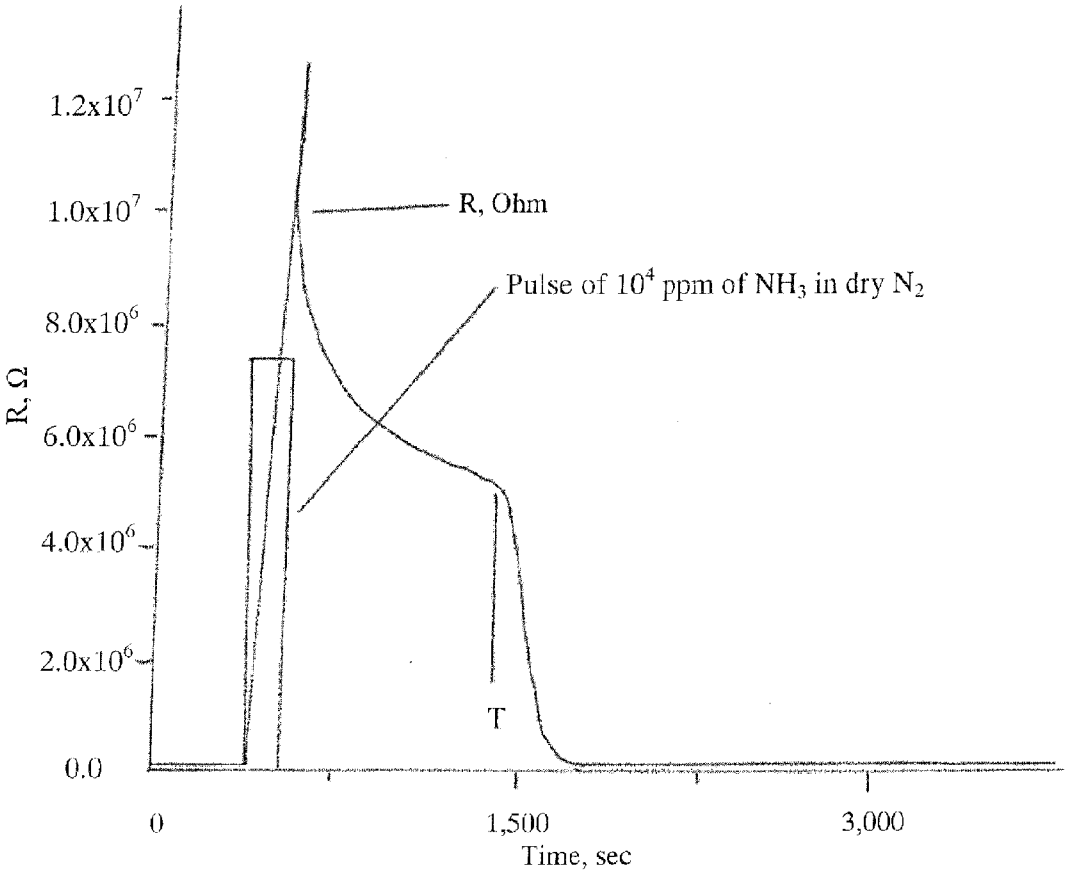
1.2x10^7
1.0x10^7
8.0x10^6
6.0x10^6
4.0x10^6
2.0x10^6
0.0
R, Ω
R, Ohm
Pulse of 10^4 ppm of NH3 in dry N2
T
0
1,500
3,000
Time, sec

Figure 5

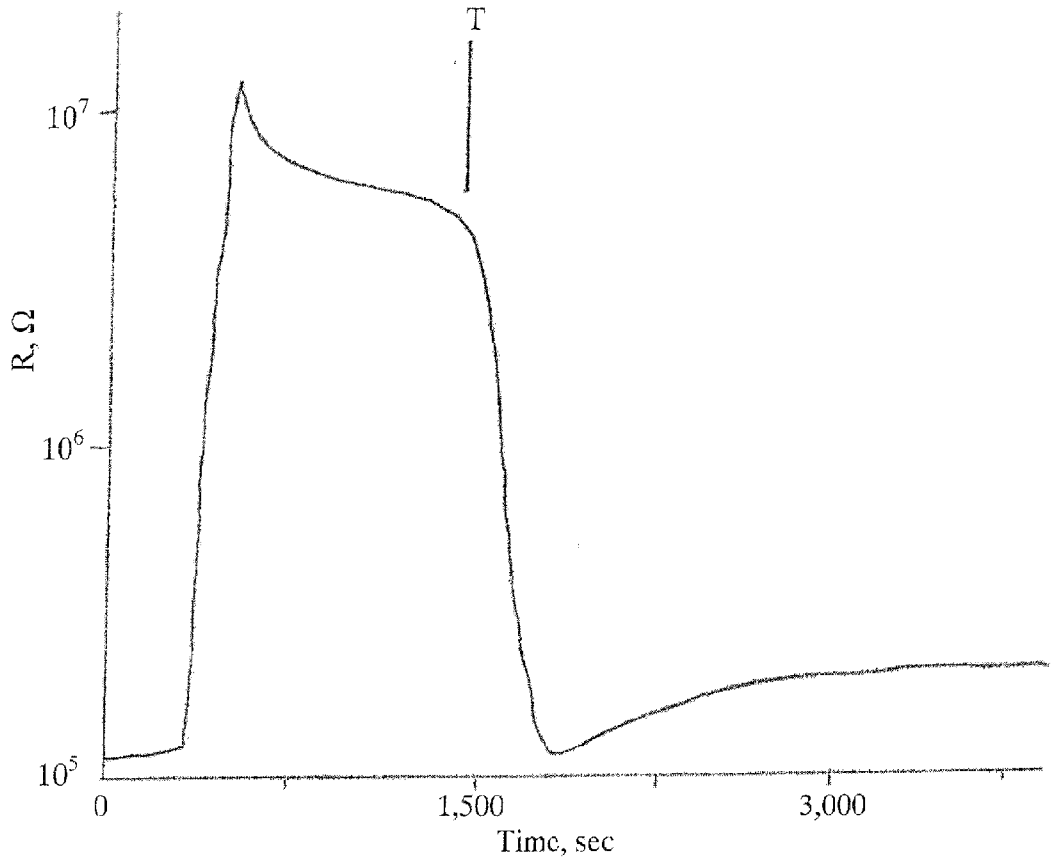

Figure 6

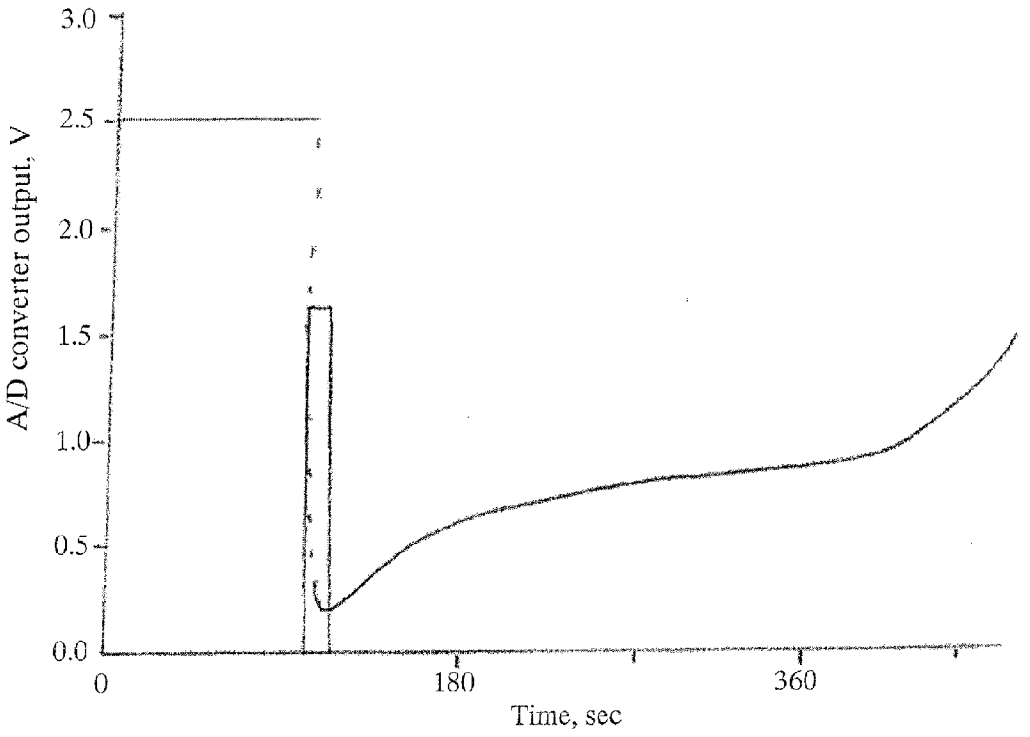

Figure 7

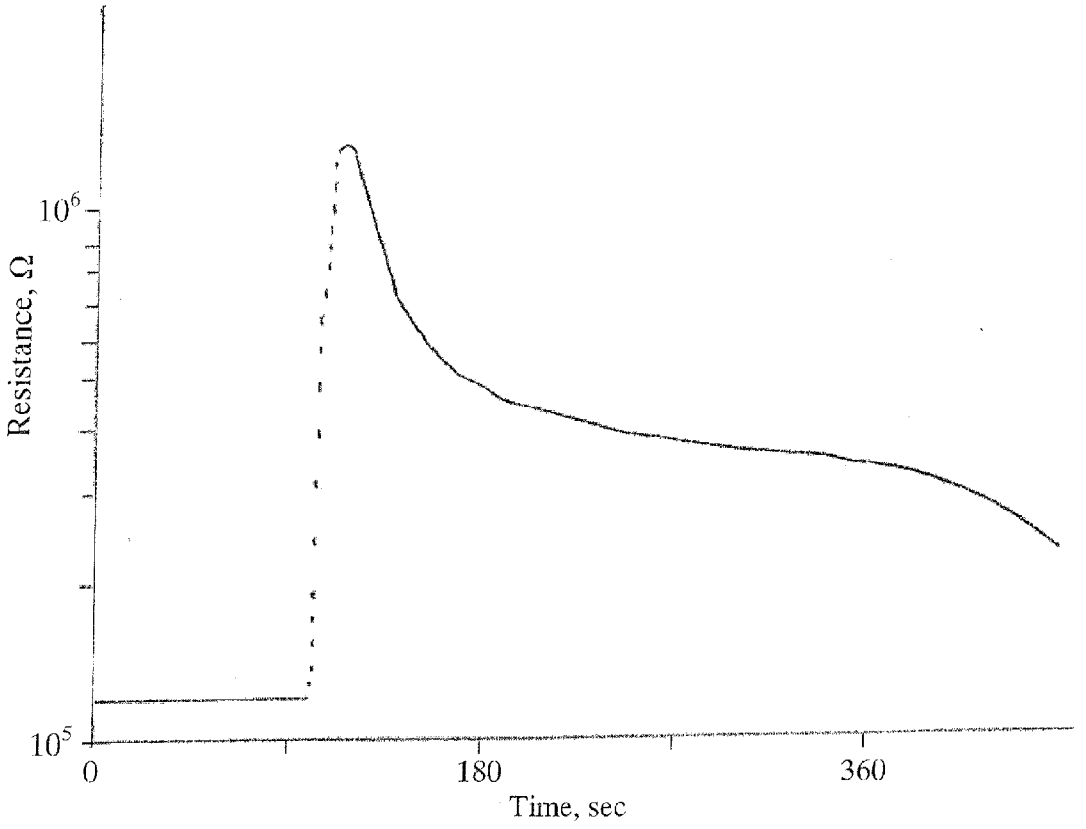

Figure 8

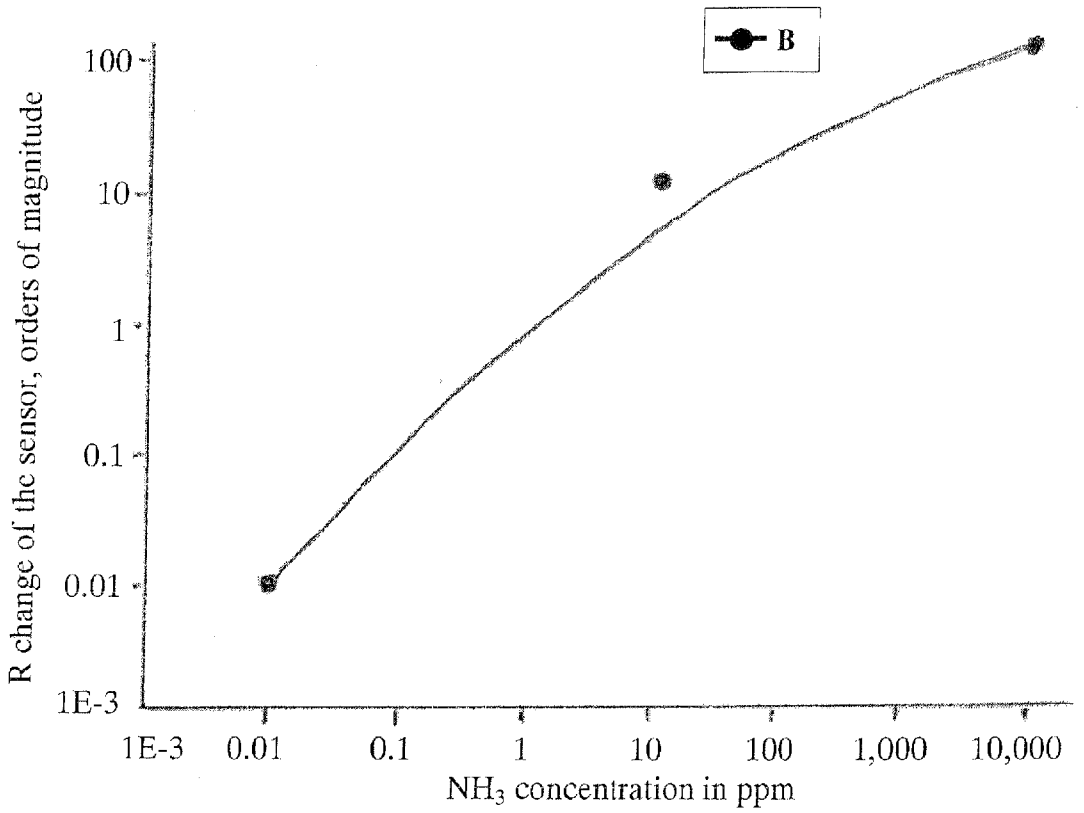
B
100
10
1
0.1
0.01
1E-3
R change of the sensor, orders of magnitude
1E-3
0.01
0.1
1
10
100
1,000
10,000
NH3 concentration in ppm

Figure 9

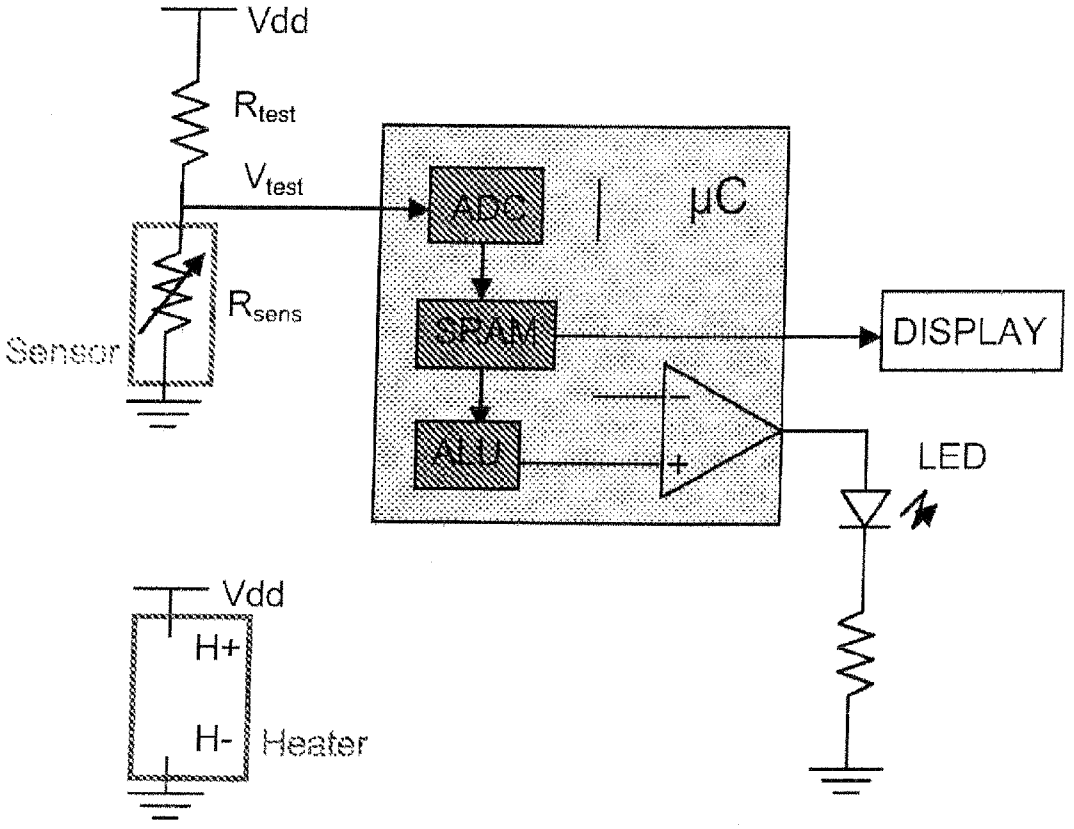
Vdd
R_{test}
V_{test}
ADC
µC
R_{sens}
Sensor
SPAM
DISPLAY
ALU
LED
Vdd
H+
H-
Heater

Figure 10

DETECTOR AND METHOD FOR DETECTION OF H. PYLORI

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority on U.S. Provisional Application Ser. No. 61/452,391 filed Mar. 14, 2011, which is incorporated by reference herein. This application also incorporates by reference U.S. Ser. No. 61/379,963 filed Sep. 3, 2010, U.S. Ser. No. 13/226,082 filed Sep. 6, 2011, and U.S. Ser. No. 61/452,507 filed Mar. 14, 2011.

FIELD OF THE INVENTION

[0002] This invention relates to a portable, hand-held, point-of-care medical diagnostic device for the detection of *Helicobacter Pylori* (*H. Pylori*) with unlabeled urea and utilizing an ammonia specific and sensitive nanosensor constructed using a polyaniline (PANI)-carbon nanotube composite.

BACKGROUND OF THE INVENTION

[0003] *H. Pylori*, a well-known pathogen of the human digestive tract, colonizes the gastrointestinal mucosa at any age, most commonly in childhood. *H. Pylori* has been associated with significant morbidity and mortality being etiologically linked to peptic ulcer disease, bleeding ulcers, gastric lymphomas (MALTOMAS) and certain forms of gastric cancer. Early diagnosis and eradication of *H. Pylori* contributes to improved health and prevention of one of the deadliest human cancers. The prevalence of *H. Pylori* in the developed world is estimated at 25%-55%, depending on demographics, and in the developing world at over 80%, the majority being children.

[0004] Detection of *H. Pylori* has been accomplished through several diagnostic modalities. These include: a) serum testing for antibodies to *H. Pylori*; b) gastrointestinal mucosal biopsies for rapid urease testing; c) culture and sensitivity of *H. Pylori* in tissues obtained through upper gastrointestinal endoscopies; d) stool testing for *H. Pylori* antigen; and e) Urea Breath Test (UBT).

[0005] Serologic testing for the detection of antibodies to *H. Pylori* is an inefficient diagnostic tool as it cannot be used to assess treatment efficacy and eradication of *H. Pylori*. Gastrointestinal endoscopy to obtain mucosal biopsies is an invasive and expensive method to detect the presence of *H. Pylori* in the gastrointestinal tract while stool testing for *H. Pylori* antigen is inefficient and cumbersome and has the lowest sensitivity and specificity of all available *H. Pylori* detection methods. 13C-Urea Breath Test is expensive and requires breath samples to be transported to a central lab facility for testing.

[0006] Urea Breath Test for the detection of *H. Pylori* is based on the ability the bacterium possesses to convert urea to ammonia and CO_2. There are two types of FDA approved, commercially-available UBTs based upon the type of urea substrate used: ^{13}C labeled Urea and ^{14}C labeled urea. ^{14}C is a radioactive isotope of carbon and the ^{14}C-UBT based on detection of $^{14}CO_2$ in breath is practically abandoned. ^{13}C is a stable non-radioactive isotope of carbon, encountered in nature and the ^{13}C-UBT based on the detection of $^{13}CO_2$ in breath is currently the gold standard breath test for the detection of *H. Pylori*.

[0007] Limitations of widespread use of ^{13}C-UBT arise from the high cost of ^{13}C-urea and of the instrument to detect $^{13}CO_2$.

SUMMARY OF THE INVENTION

[0008] The present invention solves above-mentioned shortcomings by using unlabeled urea as a substrate, by measuring ammonia in breath, instead of CO_2, and by utilizing an ammonia specific and sensitive nanosensor constructed using a polyaniline (PANI)-carbon nanotube composite.

[0009] As a result the present invention provides a highly sensitive and specific ammonia sensing device for the detection of *H. Pylori* which is inexpensive, non-invasive, portable, point-of-care and easy to operate.

[0010] The present invention eliminates the need for serologic testing for antibodies to *H. Pylori*, for gastrointestinal endoscopy to obtain mucosal biopsies and culture for *H. Pylori*, for stool-testing for the detection of *H. Pylori* antigen and for the detection of *H. Pylori* using ^{13}C-Urea Breath Test.

[0011] The present invention comprises an inexpensive, portable, hand held, point-of-care, non-invasive breath-analyzer for the detection of *H. Pylori* in adults and children by measuring ammonia in breath using a polyaniline-carbon nanotube sensor.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] FIG. **1** is a block diagram of a detector according to the invention;

[0013] FIG. **2** shows the fundamental mechanism for chemical sensing using polyaniline thin films;

[0014] FIG. **3** shows an image of the sensor in a sensor holder;

[0015] FIGS. **4**(*a*) and (*b*) show sensor output as a function of pulses of ammonia in dry nitrogen, with FIG. **4**(*a*) being a magnification of the circled area (direction of the arrow) of FIG. **4**(*b*);

[0016] FIG. **5** shows change of resistance of PANI-EB sensor in linear scale on both axes;

[0017] FIG. **6** shows resistance of the sensor in semi-log scale;

[0018] FIG. **7** shows sensor output under a pulse of 10 ppm of ammonia in nitrogen;

[0019] FIG. **8** shows sensor output under 10 ppm ammonia in nitrogen in semi-log scale;

[0020] FIG. **9** shows a graph of how the PANI-EB sensor easily detects ammonia at 10 ppb level in linear approximation; and

[0021] FIG. **10** shows electronic circuitry of a device according to the invention.

DETAILED DESCRIPTION OF AN EMBODIMENT

[0022] A preferred embodiment will be described, but the embodiment is merely one example of how to practice the invention, and the invention is not limited to this embodiment.

[0023] FIG. **1** shows a breath-analyzer which consists of mouth piece, trap, emeraldine-base polyaniline (PANI-EB) sensor, N_2 flow, and electronics. The electronics comprise an acquisition module, a memory/computation module, and a yes/no display, and a numerical display.

[0024] Technologies based on polymer thin films for electronic and chemical sensor applications are currently under development and are enabled by the availability of a special

class of polymeric materials referred to as conjugated polymers—or conducting polymers. These conducting polymers are being investigated for chemical sensor applications, and initial work focuses on the use of the emeraldine forms of polyaniline (PANI), a schematic of which is shown in FIG. **2**. Polyaniline is particularly useful as a chemical sensor for detection of ammonia vapors even at low concentrations (ppm-ppb) range. The present invention demonstrates the effect of highly conducting additives on improving the response rate and sensitivity of the sensor. Use of polyaniline thin films and their nanocomposites enables detection of low ammonia concentrations in human breath for detection of *H. Pylori*.

[0025] The fundamental mechanism for chemical sensing using polyaniline thin films is shown in FIG. **2** (See, Pure Appl. Chem. Vol. 74, No. 5 pp 857-02) where the emeraldine salt form of polyaniline is deprotonated to yield the less conducting emeraldine base form. The change in resistivity from highly conducting to practically insulating is used to gauge the extent of chemical reactivity with the nitrogen bonding sites along the PANI backbone. Exposure of the PANI emeraldine salt to ammonia produces this reversible de-doping effect allowing sensors to be re-used.

[0026] Emeraldine base polyaniline (PANI-EB) with a molecular weight of 10 k and camphor-10 sulphonic acid B were purchased from Aldrich and used as received. PANI-EB and CSA were dissolved in chloroform at a molar ratio of 1:0.5 to completely protonate the PANI backbone to produce the emeraldine salt form (PANI-CSA). Solutions were stirred for 3 days and sonicated for 15 minutes prior to preparing thin films.

[0027] Thin films were prepared using the spin casting technique on clean glass substrates with pre-patterned platinum electrodes. Glass substrates were cleaned via sonication in acetone followed by rinsing in deionized water. Polymer films were spin-cast at 1000 RPM for 45 seconds to produce films ~100-200 nm thick. A section of the thin films were removed from the Pt finger electrodes to ensure direct electrical contact during measurements by using a combination of O_2/Ar plasma in a March Plasma RIE.

[0028] FIG. **3** shows an image of the sensor in the sensor holder. The whole substrate is covered with spin-casted PANI film. The active area is located in the center of the substrate (dimension 20×10 mm) and it is 4×4 mm with four parallel planar Pt electrodes with 5 um separation and PANI film on the top.

[0029] The PANI-CSA thin film samples were characterized by measuring current flow as a function of exposure to ammonia vapors in nitrogen atmosphere at room and elevated temperatures (up to 70° C.) at fixed applied voltage. The duration of sensor exposure to ammonia and the concentration of ammonia gas varied.

[0030] The general-sensitivity of the sensor to ammonia was tested by applying relatively high concentration of ammonia in dry nitrogen. As shown in FIGS. **4-9** ammonia concentrations correspond to 10 ppm and 10,000 ppm NH_3 in N_2. The results are presented in FIGS. **4-9**.

[0031] The sensitivity of the PANI-EB sensor is significant as demonstrated by the PANI-EB sensor's ability to sense very low ppb (approximately 10 ppb) concentrations of ammonia. Use of this technology will optimize the experimental configuration of the PANI-EB sensor to meet the rigorous demands that testing in the ppb range requires. FIG. **4**(*b*) shows sensor output as a function of a pulse of ammonia in dry nitrogen of approximately 10 ppb at 100 seconds and 10^4 ppm at about 250 seconds. The initial stage of the sensor response in the circle corresponds to approximately 10 ppb, as shown in magnification in FIG. **4**(*a*), and has a sudden change of characteristic resistance in ohms from the flat baseline to a significant slope. The increase in resistance results in a drop in voltage.

[0032] FIG. **5** is a graph showing the change of resistance of the PANI-EB sensor in a linear scale on both axis in response to a pulse of 10^4 ppm of NH_3 in dry N_2.

[0033] FIG. **6** is a graph showing resistance of the sensor in semi-log scale, and shows a two order of magnitude change of the resistance at the application of 10^4 ppm NH_3 in dry N_2.

[0034] FIG. **7** shows a graph of sensor output under a pulse of 10 ppm of ammonia (NH_3) in dry nitrogen (N_2). The analog-to-digital converter (ADC) is shown (proportional to the current in the sensor, where 1 Volt corresponds to 10 $_{u}$A). The heating of the sensor up to 70° C. was introduced for the purpose of recovery of the sensor.

[0035] FIG. **8** shows a graph of sensor output under 10 ppm of ammonia (NH_3) in dry nitrogen (N_2) in semi-log scale. The resistance of the sensor in semi-log scale shows one order of magnitude change of the application of 10 ppm (NH_3) in dry (N_2).

[0036] FIG. **9** shows a graph of how the PANI-EB sensor easily detects ammonia at 10 ppb level, in linear approximation.

[0037] The PANI-EB ammonia sensor is an improvement over the MoO_3 ammonia sensor in the Urea Breath Test in the following ways:

[0038] 1. The PANI-EB sensor has higher specificity and sensitivity to ammonia than the MoO_3 sensor.

[0039] 2. The PANI-EB sensor need only be heated to 70° F. in order to return to the baseline and be ready to be re-used, while the MoO_3 sensor requires heating at 470° F. Such high heat will create problems for handling a hand-held device.

[0040] 3. The PANI-EB sensor is very easily reproduced as compared to the MoO_3 nanosensor.

[0041] FIG. **10** shows the electronic circuitry of the device. FIG. **10** shows a sensor, interface circuitry and display. A micro-controller (μC) contains the Analog-to-Digital Converter memory (SRAM), and an Arithmetic Logic Unit (ALU). The V test is a voltage proportional to the resistance of the sensor. The sensor will detect ammonia gas. If one wishes to detect gases other than ammonia, other sensors could be used. More than one sensor could be incorporated, with a switch to select connection of the sensor to the circuit for the specific gas to be detected.

[0042] Although one preferred embodiment has been shown and described, the invention is not limited to this embodiment, and the scope is defined by reference to the following claims.

What is claimed is:

1. A detector for detecting *H. Pylori*, comprising a polyaniline (PANI)-carbon nanotube composite having unlabeled urea as a substrate which detects the presence of ammonia in breath, which thereby detects the presence of *H. Pylori*.

2. The detector according to claim **1**, wherein the detector is in the form of a hand-held, portable device.

3. The detector according to claim **1**, wherein the detector further comprises an acquisition module for receiving an output signal from the PANI nanotube composite having a characteristic indicating the detected concentration of ammo-

nia and producing an output signal, a computation module connected to receive the output signal from the acquisition module and producing an output signal, and a display for displaying the results of the detector, based on the acquisition module output signal.

4. The detector according to claim **3**, wherein the display comprises a numerical display for displaying the concentration of detected ammonia.

5. The detector according to claim **3**, wherein the display comprises a visual indicator which displays the results of a threshold detection indicating the presence of *H. Pylori* above a threshold concentration.

6. A method of detecting *H. Pylori*, comprising using a polyaniline (PANI)-carbon nanotube composite having unlabeled urea as a substrate which detects the presence of ammonia in breath, which thereby detects the presence of *H. Pylori.*

7. The method according to claim **6**, further comprising using a detector in the form of a hand-held, portable device.

8. The method according to claim **6**, further comprising acquiring an output signal from the PANI nanotube composite which has a characteristic indicating the detected concentration of ammonia, using a computation module to complete the detected ammonia concentration, and displaying the results of the computation.

9. The method according to claim **8**, comprising displaying a numerical result indicating the concentration of detected ammonia.

10. The method of claim **8**, comprising comparing the detected ammonia concentration to a threshold amount, and displaying whether the amount has been exceeded, or is less than the threshold.

11. A detector for detecting the presence of ammonia in breath, comprising:

a hand-held portable housing;

a sensor in the housing for detecting the presence of ammonia in a concentration sufficiently low to detect, from a user's breath, the presence of *H. Pylori;*

an electronic circuit connected to the sensor for providing an output representative of the concentration of ammonia detected; and

an output display connected to the electronic circuit for displaying a visual signal in response to the output indicating the concentration of ammonia detected.

12. The detector according to claim **11**, wherein the detector further comprises an acquisition module for receiving an output signal from the PANI nanotube composite having a characteristic indicating the detected concentration of ammonia and producing an output signal, a computation module connected to receive the output signal from the acquisition module and producing an output signal, and a display for displaying the results of the detector, based on the acquisition module output signal.

13. The detector according to claim **11**, wherein the display comprises a numerical display for displaying the concentration of detected ammonia.

14. The detector according to claim **13**, wherein the display comprises a visual indicator which displays the results of a threshold detection indicating the presence of *H. Pylori* above a threshold concentration.

* * * * *