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A Infrared Spectroscopy method to detect ammonia in gastric juice

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Abstract

Ammonia in gastric juice is considered a potential biomarker for *H. pylori* infection and as a factor contributing to gastric mucosal injury. High ammonia concentrations were also found in patients with chronic renal failure, peptic ulcer disease and chronic gastritis. Rapid and specific methods for ammonia detection are then urgently required by the medical community. Here we present a method to detect ammonia directly in gastric juice based on Fourier Transform Infrared Spectroscopy (FTIR). The ammonia dissolved in biological liquid samples as ammonium ion was released in air as a gas by shifting the pH equilibrium of ammonium/ammonia reaction and in line detected by FT-IR set up equipped with a gas cell for the quantification. The method here developed provided high sensitivity and selectivity in ammonia detection both in pure standard solutions and in a simulated gastric juice matrix over the range of diagnostic concentrations tested. Preliminary analysis were also performed on real gastric juice samples of patients with gastric mucosal injury and with symptoms of *H. pylori* infection, and the results were in agreement with the clinical-pathological information. The whole analysis, performed in less than ten minutes, can be directly applied on the sample without extraction procedures and it assures high specificity of detection due to the ammonia fingerprint absorption bands in the infrared spectrum. This method could be easily interfaced with endoscopy instrumentation providing information in real time and enables the endoscopist to improve and integrate gastroscopic examinations.

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keywords: ammonia, gastric juice, FTIR spectroscopy, *H. pylori*, gastric mucosal injury

Introduction

Gastric juice is a colorless, watery, acidic digestive fluid that is secreted by various glands in the mucous membrane of the stomach and consists chiefly of hydrochloric acid, pepsin, rennin, and mucin. The resulting highly acidic environment in the stomach lumen causes proteins food denaturation during digestion and is able to inhibit microorganisms growth, which is helpful to prevent infection. Gastric juice is commonly thrown away during upper endoscopy and, if properly analyzed, may represent a valuable source of clinical-pathological information, especially about *Helicobacter pylori* infection and atrophic gastritis of the oxyntic mucosa (AGOM), which cannot be detected by simple endoscopic examination [1]. Invasive antral biopsies need to be applied for collecting specimen samples which are further analyzed by several methods such as histology assays, *H. pylori* culture, molecular methods (PCR) or urease test [2]. Each of these techniques is laborious and requires several hours to days before the test result is known [3]. Moreover, in patients with normal/mild endoscopic findings, endoscopists take only few antral samples or do not perform biopsies making sometimes impossible the detection of such diseases [1]. Several papers in literature have indicated ammonia in gastric juice as a potential biomarker and as a factor contributing to gastric mucosal injury. Elevated concentrations of ammonia have been frequently found in patients with chronic renal failure [4], peptic ulcer disease [5] and chronic gastritis [6]. It has been shown that ammonia accelerates apoptosis in gastric mucosa [7], inhibits proliferation and cell cycle progression at S-phase [8], impairs cell migration and proliferation of gastric mucosa [9]. Ammonia is mainly produced by *Helicobacter pylori* who may contribute to gastric mucosal injury. H. pylori has several adaptations for an acid milieu of the stomach. One of them is the urease enzyme, which converts urea into ammonia and carbon dioxide to establish a locally neutralizing surrounding against penetrating acid [10]. This is one of the features that make it possible for the bacterium to survive in the human stomach and enables the colonization of gastric mucosa. Ammonia is transformed to $NH4^+$ ion (ammonium) and the relative concentration of these two forms is pH dependent. Gastric juice ammonia has been successfully related to *H. pylori* infection

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and the severity of gastritis in several studies [3, 6, 11-14]. Medical community is considerably interested in ammonia analyzers that can be applied for measuring ammonia levels in gastric juice for the diagnosis of certain diseases especially related to *H. pylori* infection. Development of diagnostic tests that may be performed at the time of the endoscopy and rapidly interpreted by the endoscopist is important. At the present, quantitative estimation of ammonium in biological fluids is usually based on spectrophotometric procedures, potentiometric ion selective electrodes or on amperometric enzyme electrodes [15-17]. Although many of these procedures have been reported and improved over the years, all these methods suffer some limitations in terms of electrode low sensitivity, low selectivity in a complex matrix, enzymes expensive cost and frequent or long calibration procedures.

The method developed in this work is a cost effective, in-line and no contact analysis which is able to detect ammonia present in gastric juice by analyzing the saturated vapor. Ammonium (liquid phase) in gastric juice is forced to be released as ammonia (gas phase) in air by varying the pH of the solution which moves the equilibrium of the reaction to the gas phase. Ammonia in gas phase has a typical absorption bands in the infrared spectrum that can be rapidly detected in real time. The goal of this study is to determine the sensitivity of this FTIR based method over the diagnostic ammonia concentrations range and to evaluate the role of interferents in a gastric juice simulated matrix. Moreover, gastric juices of patients with gastric mucosal injury and with symptoms of *H. pylori* infection were analyzed in order to assess the feasibility of this test in a real sample.

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Material and Methods

Reagents

Ammonium chloride (NH_4Cl), Sodium chloride (NaCl), hydrochloric acid (HCl, 37%) and sodium hydroxide (NaOH) were purchased from Carlo Erba (Milan, Italy). Pepsin 1:10000 was purchased from Farmalabor (Farmacia Specchiulli). All chemicals were of analytical grade and used without further purification.

Preparation of standard solutions

Ammonium chloride stock solution was prepared by accurately dissolving 0.943 g of NH_4Cl in 1 l of deionized (DI) water to reach a concentration of 943 ppm. Ammonium chloride standard solutions were prepared by subsequent dilutions of the stock solution in DI water to reach the following concentrations: 153, 70, 60, 50 and 17 ppm. Pure ammonium chloride standards were used to set up the analytical procedure. Aliquots of the NH_4Cl standards were mixed in a 9:1 ratio with ISA solution (Ionic strength adjuster, NaOH 20% in DI water), mixed with a magnetic stirrer and the evolved gas phase was analyzed by Fourier Transform Infrared Spectroscopy equipped with a 6.4 m gas cell. Setup and measurements details are explained in the paragraph Measurement Apparatus.

Simulated gastric fluid (SGF) was prepared by dissolving 2.0 g of sodium chloride and 3.2 g of purified pepsin, that is derived from porcine stomach mucosa, with an activity of 800 to 2500 units per mg of protein (or 250 mg of 1:10000 pepsin), in 7.0 ml of hydrochloric acid and sufficient water to make 1 l (NOTE – Pepsine activity is described in the Food Chemical Codex specification under General Tests and Assays). This test solution has a pH of about 1.2. Specification of 1:10000 pepsin means 1g pepsin contains 10000 pepsin units, we can calculate that 1mg contains 10 units. If the pepsin is not purified (when it is not marked "purified"), it consists of protease, amylase and lipase. So, when it is not purified the units calculated is, indeed, a smaller number. *NF Unit*: Pepsin digests

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not less than 3000 and not more than 3500 times its weight of coagulated egg albumin. (1 pepsin unit will digest 3000 units coagulated egg albumin at 52°C, pH 2-3, no time involved.)

Ammonium chloride standard solution in simulated gastric fluid was prepared by dissolving 0.943 g of standard in 1 l of SGF to reach a concentration of 943 ppm. Ammonium chloride standard solutions were prepared by subsequent dilutions of the stock solution in SGF to reach the following concentrations: 153, 60, 50 and 17 ppm respectively. Aliquots of standards solutions in SGF were mixed with ISA (9:1 ratio) and the evolved gas phase was analyzed by FT- IR equipped with a 6.4 m gas cell. Setup and measurements details are explained in the paragraph Measurement Apparatus.

Gastric juice samples

Real gastric juice samples were provided by the Gastroenterology Division of Mauriziano Hospital (Turin, Italy). Three different samples were provided: sample A was obtained from a diluted solution used for endoscope cleaning. This sample was chosen as negative control because it contains a very low concentration of ammonia as well as a healthy patient. Sample B was from a celiac patient with symptoms of *Helicobacter pylori* infection and Sample C from a patient with stomach ulcer. All these samples have been stored in sterile tubes at 4° C in the dark and analyzed in the same day of the endoscopy.

Measurement Apparatus

The measurement apparatus is described in figure 1. Briefly, 4.5 ml of sample solution (NH_4Cl standard solution in water/SGF or real gastric juice samples) are injected in a two neck round bottom flask with magnetic stirring to continuously mix the solution. The flask is immersed in a water bath at 30° C to keep the temperature stable. One neck is used for solution injection and is subsequently safely sealed with a cap. The other one is connected through a Teflon tube to a MARS Series long path gas absorption cell for FTIR gas analysis which is located and aligned inside the FTIR spectrophotometer. A stopcock valve (valve n.1 in the scheme) is positioned between the

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flask and the gas cell to regulate the communication between these two compartments. The MARS series cell gas has an optical path of 6.4 m and a volume of 0.75 l assuring high sensitivity with potential gas detection in the ppb range. Internal body of the cell contains gold reflecting mirrors and the internal surface is covered with passivated stainless steel to avoid corrosion and contaminations when a corrosive gas is used. A thermocouple connected to a programmable temperature control is located inside the cell which is externally enveloped with an insulator sheet to keep the temperature constant at 30° C. On the upper side of the gas cell two separate valves allow to control the inlet and the outlet of the gas. The inlet is connected with the Teflon tube to the flask while the outlet is connected to a vacuum pump which is able to create a depression between the gas cell compartment and the solution containing flask. As soon as the standard solution is injected into the flask, a depression between these two compartments is applied by opening the inlet and outlet valves on the gas cell while keeping the stopcock valve above the flask closed. When a pressure of about 1013.25 Pa (10^{-2} atm) is achieved, the outlet valve is closed and the stopcock valve on the top of the flask is opened allowing the gas phase to be injected inside the gas cell. An infrared spectrum of the gas sample is then collected and it is used as a background for the FTIR measurement. When the FTIR background is collected, the stopcock valve on the flask is closed and the outlet valve is opened in order to remove the gas sample from the cell by means of the pumping system. The optical path is further cleaned before and after every measurement by injecting nitrogen gas through the stopcock valve n.2 which allows to remove any gas residues from the system. For ammonia detection, 0.5 ml of ISA solution (Ionic strength adjuster, NaOH 20% in DI water) is pipetted inside the flask and allowed to react for 5 minutes. The ISA solution changes the pH of the standard solution to alkaline values (pH around 11) and promotes the transition of ammonium (liquid phase) into ammonia (gas phase). Using the same measurement procedure just described, an infrared spectrum is collected and the ammonia can be quantified.

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FTIR Measurements

The infrared spectra of gas samples were collected using a Nicolet Nexus Thermo Fischer spectrophotometer equipped with a DTGS detector in transmission mode; 64 scans with a resolution of 4 cm^{-1} were registered for each spectrum.

MARS Series long path gas absorption cell (Gemini scientific instruments) was located into the main compartment of the instrument and an alignment procedure was performed. Background spectra were collected on every samples before adding ISA solution. A cleaning procedure is applied after every measurement using nitrogen purging and vacuum by means of a piston pump, as described in measurement apparatus.

Calibration procedure

Quantitative analysis of levels of ammonia in FTIR measurements is based on the Lambert-Beer's law which relates the absorbance at a specific wavelength with the standard concentration. For each standard solutions of ammonium chloride, both in pure water or in SGF, the peak height of the absorption band at 966 cm-1 (N-H symmetric deformation of ammonia) was calculated. Hence, a regression curve was fitted to the intensities, providing therefore the calibration curve of the spectrophotometer for the ammonia, as the basis for subsequent quantification analyses. A weighted least-squares (WLS) regression was applied to the data, that is able to deal with uncertainty in the values of the dependent variable, i.e., the absorbance values. The algorithm used for the WLS regression is a MATLAB®-based tool for calibration problems (Calibration Curves Computing – CCC Software, Release 1.2), recently developed at INRIM in the framework of the Joint Research Project "Novel mathematical and statistical approaches to uncertainty evaluation", within the European Metrology Research Program.

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Results and Discussion

High concentrations of ammonia have been found in several gastric diseases, especially the ones related to *H. pylori* infection. New methods for detecting ammonia in gastric fluid are then required in order to make rapid diagnosis and to prevent the aggravation of such diseases. The proposed method represents an alternative way to detect ammonia in biological fluids and in particular in gastric juice. Infrared spectroscopy is a very sensitive and selective technique for gas detection since these molecules have strong absorption bands related to the roto-vibrational structure of the gas molecules in the medium infrared region that can be rapidly identified due to their specific fingerprint. In order to develop a method to detect ammonia (gas) in gastric juice by FTIR spectroscopy, the ammonium ions in solution must be released in air as gas phase. The ammonium/ammonia equilibrium is very well known in chemistry and the direction is governed by the pH of the solution. Ammonium hydroxide is a weak base that is partially ionized in water according to the equilibrium:

$$NH_3(gas) + H_20 \leftrightarrow [NH_4OH] \leftrightarrow NH_4^+ + OH^-$$

When the pH is equal to the pKa (9.25 pH), half of the ammonia will be un-ionized (NH_3) and half will be ionized (NH_4^+). At pH 10.25 and 11.25, 90% and 99% of the ammonia will be un-ionized, respectively. The volatility of ammonia increases with increasing pH; therefore, it volatilizes freely from solution at high pH values. This condition can be forced by adding a proper amount of sodium hydroxide (ISA solution) in the gastric juice to shift the pH to a value around 11. As soon as the ammonia evolves from the solution in the gas phase and when it reaches the vapour saturated condition, the gas phase can be rapidly injected into the FTIR gas cell (see Fig. 1 and Measurement apparatus in Material and methods for details) and the ammonia can be specifically detected by functional group identification in the infrared spectrum.

Figure 1

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A standard solution of 943 ppm ammonium chloride was first tested to set up the procedure and to carry out infrared qualitative analysis of ammonia. A FTIR spectrum of the pure solution was first collected without adding ISA in order to register the signal background. As Fig. 2a shows, this spectrum, in the range of 800 cm^{-1} to 4000 cm^{-1}, is mainly characterized by water absorption bands with O-H stretching vibrations at 3600-3800 cm^{-1} , H-O-H bending vibrations at 1500-1700 cm^{-1} and C-O stretching vibration at 2350 cm^{-1} due to the presence of carbon dioxide (CO_2). No other signals can be distinguished throughout the spectrum. However, as soon as the ISA solution is added into the flask to move the reaction equilibrium from ammonium to ammonia, specific ammonia absorption bands show up. FTIR spectra in Fig. 2b, c, d clearly show in the three different spectral regions the presence of the N-H symmetric stretching vibration at 3300 cm^{-1} (ν_1), the degenerate deformation at 1640 cm^{-1} (ν_4) and the symmetric deformation with a double peak at 966 and 934 cm^{-1}(ν_2), respectively [18].

Figure 2

In order to investigate the sensitivity and the dynamic range of the FTIR based method, ammonium chloride standard solutions with a concentration of 17, 50, 60, 70, 153 ppm respectively were then analyzed. Since the absorption band at 966 cm^{-1} has the highest intensity and is free of water interference (Fig. 2d), it was the frequency band selected signal for ammonia quantification. As Fig. 3a shows, the ammonia absorption band at 966 cm^{-1} raises proportionally with the ammonium concentrations.

Figure 3

Calibration of the infrared equipment was performed by using the Lambert-Beer's law that is expressed by the equation:

$$\mathbf{A(\nu)=\varepsilon(\nu)l\,C}$$

where

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A(ν) is the absorbance at wave number ν ,

ε(ν) is the molar absorptivity at wave number ν ,

l is the path length,

C is the molar concentration.

Thus, it was possible to correlate the absorbance values at 966 cm^{-1} with the ammonium chloride concentrations (Fig. 3b). The relative standard uncertainty associated with the absorbance values (reported as *y* error bars in Fig. 3b) was considered equal to 10 % in the considered ammonia range. This value was evaluated on previous experiments aimed at assessing the reproducibility of the FT-IR signals. Notice that calibration uncertainty associated with the standard concentrations were proved to be negligible with respect to the absorbance variability, hence they were not take into account in the present analysis. A 1^{st} order polynomial fit $y = a + bx$ was used for fitting the data (Fig. 3b), and the relevant normalized χ^2 value (i.e., the sum of the weighted squared residuals normalized by the number of degrees of freedom) showed a high goodness of fit for ammonia in water, being lower than the expected unit value.

According to the literature, the 60 ppm concentration of ammonia has been established as a cut off to determine the infection of *H. pylori*. This threshold value has been calculated by Tucci et al., 2007 [1] during the validation of the Mt 21-42 device, an ion selective electrode that measures ammonia in gastric juice. This analysis have stated 60 ppm of ammonia as a cut off value for patients infected with *H. pylori* by comparing the obtained results with traditional analytical methods such as histology, breath test analysis and serological test. In order to assess whether the FTIR based method can be potentially used for ammonia detection and for the identification of patients infected with *H. pylori*, concentrations around the threshold value of 60 ppm were chosen. The results shown in Fig. 3a-b have indicated that our methodology display high sensitivity at low concentration of ammonia such as 17 ppm and that is able to discriminate ammonia concentration

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with a good resolution around the cut off value of 60 ppm with a linear correlation along the whole range tested.

After the method was calibrated with pure standard solutions in water, we decided to test ammonia detection in a simulated gastric juice matrix. SGF solution contains several interferents frequently found in a real gastric juice sample such as hydrochloric acid, pepsin, enzymes and ions. Specific amounts of ammonium chloride were spiked into the simulated gastric juice to calibrate the FTIR based method in presence of such interferents. The diagnostic concentrations tested were 0, 17, 50, 60 and 153 ppm, respectively. FT-IR spectra were collected for these standards before and after adding ISA solution and the absorbance values were plotted over the cited concentrations (Fig. 4).

Figure 4

As in the case of ammonium chloride standard solutions, a relative standard uncertainty equal to 10% was associated with the absorbance values (*y* error bars in Fig. 4b), and calibration uncertainty in the standard solutions was negligible. In this case, a 2nd order polynomial fit $y = a + bx + cx^2$ was used for fitting the data (Fig. 4b), and a very good corresponding χ^2 value showed a high goodness of fit for ammonia in SGF. Estimates of the fitting parameters *a*, *b* and *c* and associated covariance matrix were calculated according to the WLS procedure. From such results, it was straightforward to obtain the analysis curve $x = \left(b - \sqrt{b^2 + 4c(-a + y)}\right)/(-2c)$, by simply inverting the calibration curve. Therefore, given a new measured absorbance value *y*, corresponding to an unknown concentration *x* of ammonia, an estimate for such measurand can be derived by the formula above; the corresponding associated uncertainty can be obtained by applying to the analysis curve either the law of propagation of uncertainty [20, 21]. A Monte Carlo simulation was performed according to latter choice, in which parameter estimates *a*, *b* and *c* were considered distributed according to a multivariate normal distribution with covariance matrix equal to that determined during the calibration process, and intensity *y* distributed as a normal distribution with standard deviation equal to the repeatability uncertainty typically associated with *y*.

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Comparing these results with those obtained in pure standard solutions, it was shown that absorbance values of ammonia from a gastric juice simulated matrix are lower than those obtained from standard water solutions. Moreover, the linearity is no more preserved. It is possible to infer that a latency phase is maintained from 0 to 40 ppm while above this value a linear response is starting again. Deviation from linearity is probably caused by interferents in solution. Since several charged molecules such as proteins and ions are present in the simulated gastric juice and they are able to interact with ammonium, the shift of the ammonium/ammonia equilibrium could be partially slowed down by association/dissociation and interaction phenomena with the chemical species in solution. This behavior is indeed more evident at low concentrations of ammonium chloride where a latency phase is observed. At higher concentrations, instead, more ammonium molecules are free of interactions with the other species in solution and their release in air as ammonia occurs faster, leading to a linear increase of the signal. Although the linearity is no more preserved and the sensitivity is impaired at lower concentrations, the FTIR based method is still able to sense the concentrations of ammonia in the whole range tested and to discriminate around the cutoff value of 60 ppm. The relative expanded (k = 2) uncertainty associated with an estimate x of the concentration of ammonia in a concentration range from 0 up to 153 ppm appeared to be lower than 13%. Thus, the trend line obtained in Fig. 4b was used as a calibration curve in order to quantify ammonia concentrations in real samples. Gastric juices of patients who underwent to upper gastrointestinal endoscopy were analyzed by FT-IR methodology and the results were compared to the clinical-patological information. As a preliminary study on real samples, three different kind of gastric juices were used for the analysis. Gastric Juice A was obtained from a diluted solution used for endoscope cleaning. This sample was chosen because it contains a very low concentration of ammonia and represents a negative control similar to an healthy patient. Gastric Juice B was from a celiac patient with symptoms of *H. pylori* infection. The last sample (Gastric Juice C) was obtained from a patient with stomach ulcer disease. As Fig. 5 shows, FTIR analysis on real gastric juice samples registered different absorbance values of the N-H symmetric deformation at 966 cm^{-1}, with

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a lower absorbance for the negative control (0.0015 Abs. for Sample A) and higher values for the patients (0.0098 and 0.03 Abs. for Sample B and C, respectively) which means that different ammonia concentrations were measured by our FTIR based method. Using the calibration curve shown in Fig. 4, the ammonia concentrations of these samples were quantified and compared with the clinical-patological information (table 1).

Figure 5

Table 1

In sample A, a concentration of only 30 ± 5 ppm of ammonia was registered which is consistent with a negative control sample as well as a healthy patient. In sample B and C, instead, high values of ammonia were registered by FT-IR methodology which can be related to the critical clinical conditions of the patients, as it is shown in Table 1. In the celiac patient (Sample B) with symptoms of H. pylori infection, a concentration of 97 ± 13 ppm was measured which is higher than the cutoff value of 60 ppm indicated by Tucci et al., to determine the infection of *H. pylori*. Therefore, such amount of ammonia could be reasonably related to the presence of the infection. In the latter case (Sample C), a very high concentration of ammonia, more than 153 ppm, was detected, suggesting that the patient has a severe and critical stomach condition which is in agreement with the clinical information (Table 1). Also in this case it could be inferred an infection of *H. pylori* but histological analysis was not performed on this sample. Even if these analysis only give preliminary information and a complete validation of this methodology in real samples is still missing, these results demonstrated the application of FTIR spectroscopy for the quantification of ammonia in gastric juice. Moreover, since high concentrations of ammonia in blood, saliva and urine were related to inflammatory states, the FTIR method here proposed could be successfully used as screening test for the analysis of biological fluids in the medical field.

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Conclusion

Ammonia is an interesting biomarker that can be found in several biological fluids such as saliva, urine, blood and gastric juice. In the latter case, elevated concentration of ammonia were related to *H. pilori* infection and in general to gastric mucosal injury. In this work we presented a new method to detect ammonia in gastric juice based on FTIR spectroscopy that could be potentially applied to other biological fluids. The ammonia dissolved in biological liquid samples as ammonium ion can be released in air as a gas, by shifting the equilibrium of ammonium/ammonia reaction which is mainly governed by the pH of the solution. Ammonia detection was performed by FTIR spectroscopy which guarantees high sensitivity and specificity both in pure standard solutions and in a simulated gastric juice matrix over the range of diagnostic concentrations tested. Real gastric juice samples were also analyzed and the obtained results demonstrated the variability associated to the patients with clinic-pathological diseases. Our method proved to be very rapid because it does not require extraction procedures, the total analysis time takes less than 10 minutes and it is very easy to be calibrated. Moreover, since ammonia has a specific fingerprint in the infrared spectrum, false positive responses can be minimized.

This innovative method could be easily interfaced with endoscopy instrumentation providing information in real time and enables the endoscopist to improve and integrate gastroscopic examinations by comparing the outcome of visual inspection and chemical testing.

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FIGURE LEGENDS

Figure 1 – FTIR gas analysis setup

Figure 2 – (a) FTIR spectrum of a 943 ppm NH_4Cl solution in water (gas phase). (b, c, d) FTIR spectra of a 943 ppm NH_4Cl water solution (gas phase) before (dotted line) and after (black line) adding ISA (20% NaOH in water) in the range 3500-3150 cm^{-1} (b), 2000-1400 cm^{-1} (c) and 1100-850 cm^{-1} (d).

Figure 3 - (a) FTIR spectra of NH_4Cl water solutions (gas phase) after adding ISA: 17, 50, 60, 70 and 153 ppm. (b) Calibration curve of NH_4Cl standards in water obtained by plotting the Absorbance at 966 cm^{-1} vs NH_4Cl concentration.

Figure 4 - (a) FTIR spectra of NH_4Cl solutions in simulated gastric fluid (gas phase) after adding ISA: 0, 17, 50, 60, and 153 ppm. (b) Calibration curve of NH_4Cl standards in simulated gastric fluid (SGF) obtained by plotting the Absorbance at 966 cm^{-1} vs NH_4Cl concentration.

Figure 5 – FTIR spectra of real gastric juice samples (gas phase) after adding ISA obtained from an healthy patient (Sample A), a patient with symptoms of *H. pilori* (Sample B) and a patient with stomach ulcer (Sample C).

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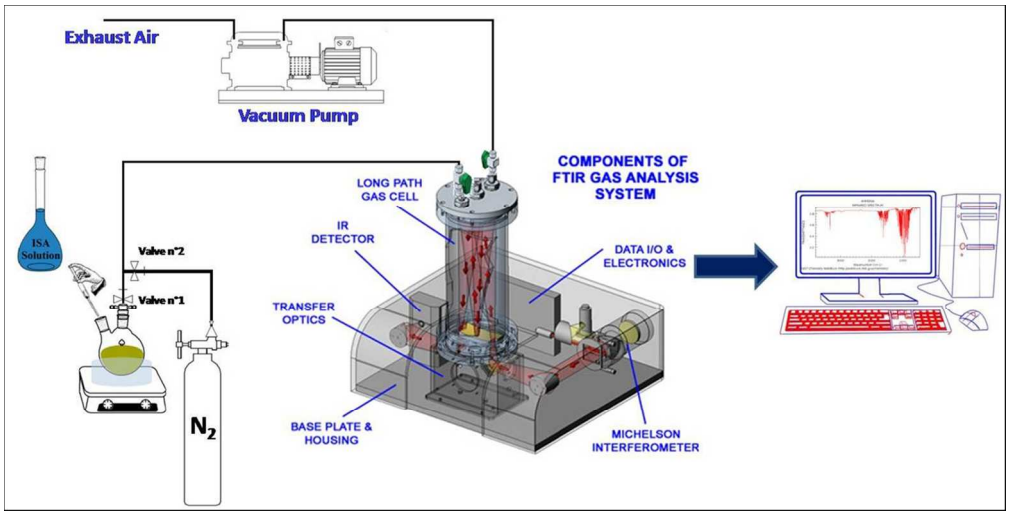

Figure 1 – FTIR gas analysis setup
244x123mm (150 x 150 DPI)

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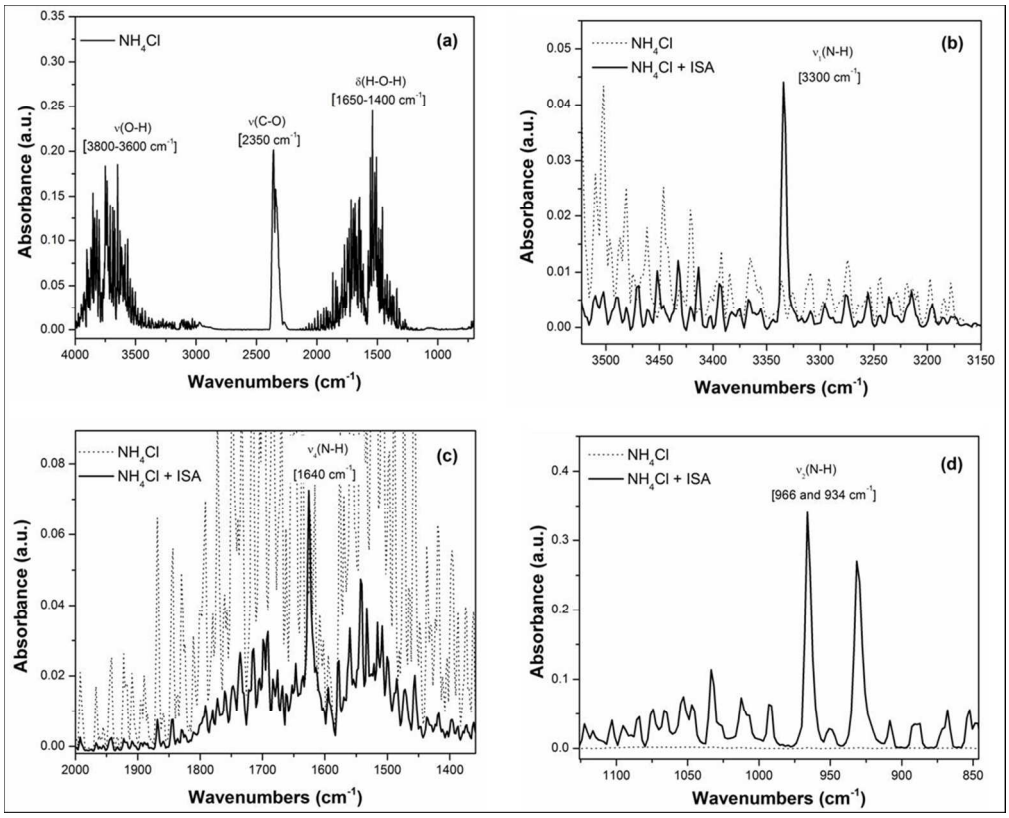

Figure 2 – (a) FTIR spectrum of a 943 ppm NH_4Cl solution in water (gas phase). (b, c, d) FTIR spectra of a 943 ppm NH4Cl water solution (gas phase) before (dotted line) and after (black line) adding ISA (20% NaOH in water) in the range 3500-3150 cm^{-1} (b), 2000-1400 cm^{-1} (c) and 1100-850 cm^{-1} (d).
215x173mm (150 x 150 DPI)

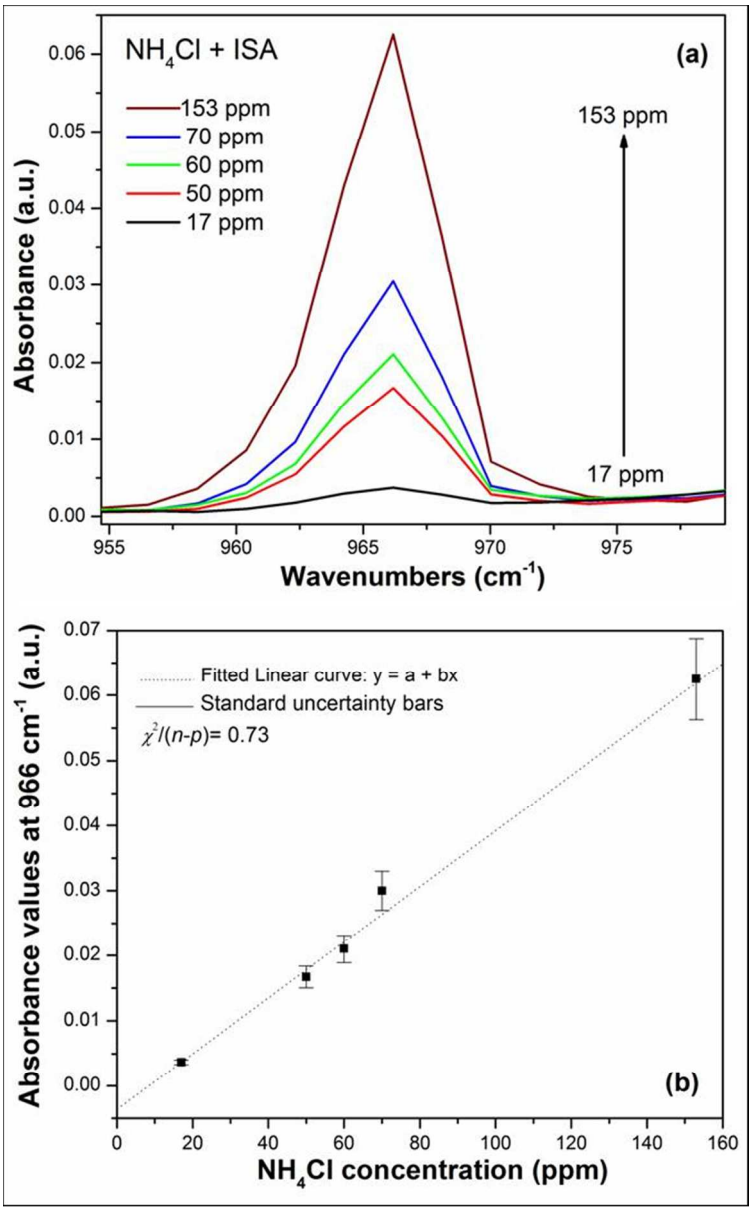

Figure 3 - (a) FTIR spectra of NH_4Cl water solutions (gas phase) after adding ISA: 17, 50, 60, 70 and 153 ppm. (b) Calibration curve of NH4Cl standards in water obtained by plotting the Absorbance at 966 cm^{-1} vs NH_4Cl concentration.
113x183mm (150 x 150 DPI)

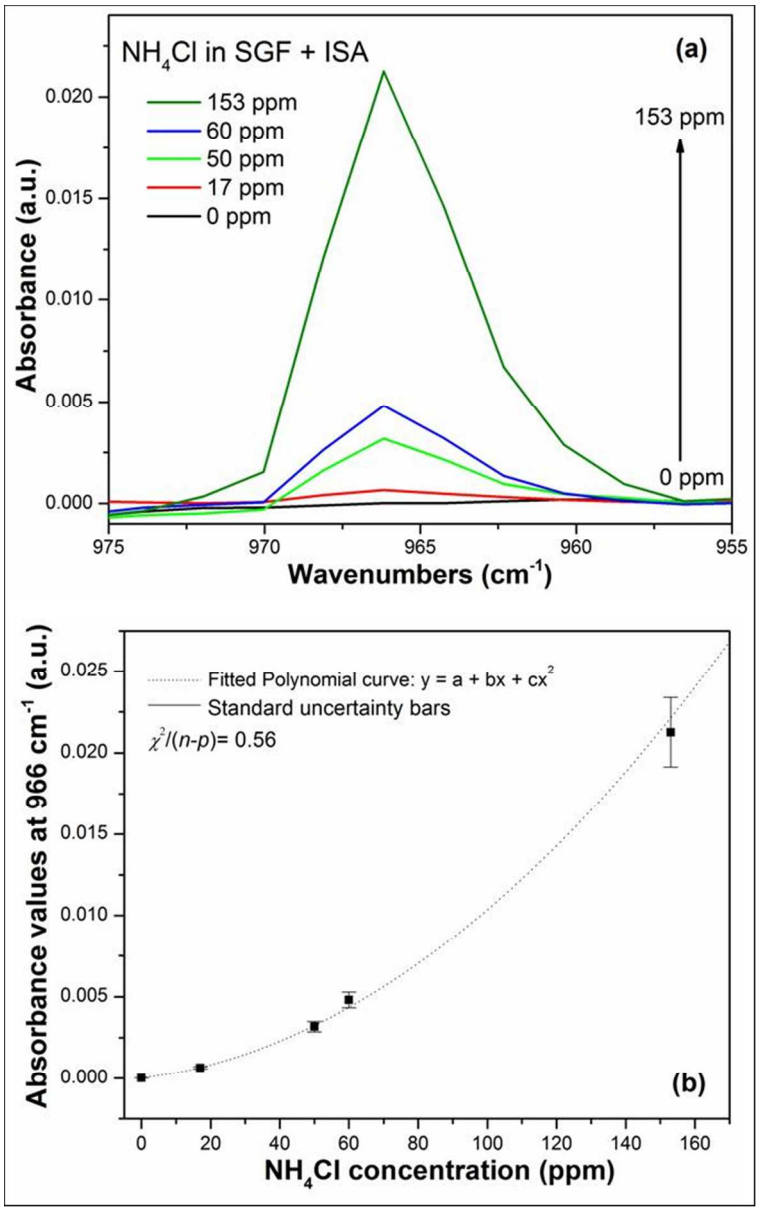

Figure 4 - (a) FTIR spectra of NH_4Cl solutions in simulated gastric fluid (gas phase) after adding ISA: 0, 17, 50, 60, and 153 ppm. (b) Calibration curve of NH_4Cl standards in simulated gastric fluid (SGF) obtained by plotting the Absorbance at 966 cm^{-1} vs NH_4Cl concentration.
114x183mm (150 x 150 DPI)

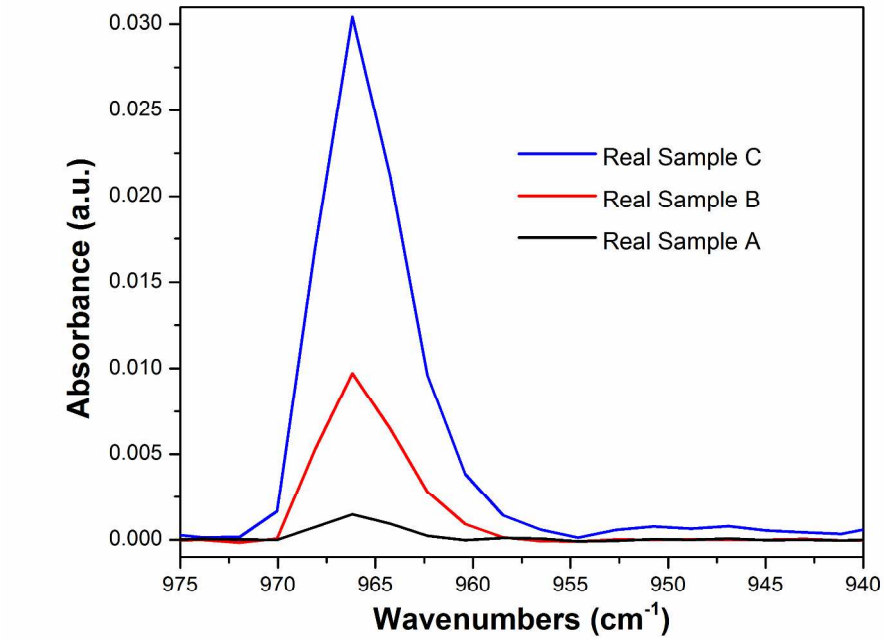

Figure 5 – FTIR spectra of real gastric juice samples (gas phase) after adding ISA obtained from an healthy patient (Sample A), a patient with symptoms of *H. pilori* (Sample B) and a patient with stomach ulcer (Sample C).
273x210mm (300 x 300 DPI)

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Table 1 Analysis of real gastric juices

Sample	Clinical-Pathological Information[a]	FTIR Quantification[a]	Expanded Uncertainty (U) (k = 2)[b]
A	Healthy Patient	NH_4^+ = 30 ppm	± 5 ppm
B	Symptoms of H. *pilori* infection	NH_4^+ = 97 ppm	± 13 ppm
C	Stomach Ulcer	NH_4^+ > 153 ppm	N.A.

[a] Ammonia quantification by FTIR in real gastric juices is based on the calibration curve in Fig.4b.
[b] Expanded Uncertainty (U) with a coverage factor k = 2 is reported for ammonia amounts in FTIR analysis.

Table 1 - Analysis of real gastric juices
189x121mm (96 x 96 DPI)

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