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Fluorometric Determination of Gastric Acidity with 2-Fluorenecarboxaldehyde Hydrazone

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Fluorenecarboxaldehyde hydrazone (FCH) was synthesized as a new fluorescent reagent for the determination of acidity. FCH had no fluorescence in itself, however, it strongly fluoresced under acidic conditions at excitation maximum of 392 nm and emission maximum of 447 nm, respectively. It showed good correlation with pH in the range from pH 0.60 to pH 3.60 in strong acids. As an application, the acidity of gastric juice in rats and humans was determined. In comparision with pH-metry, the acidity measured by the developed method showed generally increased values of about 0.7-1.3 unit and 0.8-1.2 unit in rats and humans, respectively. However, these results had statistically close correlation with those of pH-metry and the correlation coefficients were 0.806 and 0.887 in rats and humans between two methods.

Key words: Gastric juice acidity, pH, Fluorometric assay, 2-Fluorenecarboxaldehyde hydazone (FCH)

INTRODUCTION

In the diagnostic point, the measurement of gastric acidity is an important tool for checking diseases such as chronic gastritis, achlorhydria, hypochlorhydria, pancreatic neoplasm and peptic ulcer.

To date, various methods for the assay of gastric acidity have been reported including the use of a pH-meter (Feldman *et al.*, 1980; Khan *et al.*, 1981; Johnson *et al.*, 1958), litmus paper (Farinati *et al.*, 1987) and titration (Cantor *et al.*, 1990; Soldani *et al.*, 1988). These methods have some disadvantages as follows. A certain amount of sample is required for the immersion of an electrode in extragastric pH-metry, visual error in litmus paper methods is not negligible and titration methods need procedural steps. As a more recent approach, intragastric pH measurement with electrodes, mainly glass electrodes, is used (Moore and Scarlata, 1965; Moore, 1963; Moore, 1968). However, technical difficulties such as proper placement of the electrode and pH fluctuations by swallowed air limit this technique mainly to research work. Occasionally, intragastric titration is used (Holtman *et al.*, 1990; Maxwell *et al.*, 1984). It is involved with intragastric acid-base titration by infusion of sodium bicarbonate into the stomach to maintain a constant pH of 7.0. In this method, an induced unphysiological condition caused by the neutralization of intragastric hydrochloride is given intentionally.

As indirect methods, the pH of gastric juice is determined by the plasma secretin concentration (Kehl *et al.*, 1986), the application of Handerson-Hasselbalch equation (Fiddian-Green *et al.*, 1983) and the applied potential tomography (Avill *et al.*, 1987; Baxter *et al.*, 1988). However, these methods do not show good correlation with the methods mentioned above.

In this report, we describe a new fluorometric method using 2-fluorenecarboxaldehyde hydrazone (FCH). It fluoresces quantitatively under acidic conditions (Choi *et al.*, 1991). We applied this characteristic to the determination of gastric acidity in rats and humans.

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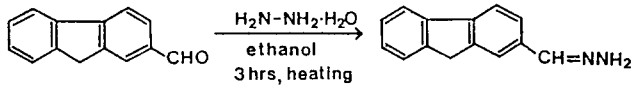
MATERIALS AND METHODS

Instruments

A pH-meter with a single electrode (pH-meter; model 7, Coming Sci. Co., U.K., electrode; ORION ROSS Combination pH No. 529) was used for pH-metry. Sonicator (Dong-Yang Sci. Co., Korea) was used for pretreatment of samples. The fluorescence spectra were obtained using a spectrofluorometer (JASCO mo-



2-Fluorenecarboxaldehyde — 2-Fluorenecarboxaldehyde hydrazone

Scheme 1. Synthesis of 2-FCH.

del FP-777, Japan).

Reagents

FCH was synthesized from 2-fluorenecarboxaldehyde (Aldrich Chem. Co., Milwaukee, U.S.A.) and hydrazine monohydrate (Yakuri Pure Chem. Co., Osaka, Japan) according to the method described by Choi et al. (Scheme 1). Hydrochloric acid (Wako Pure Chem. Industries, Ltd., Osaka, Japan) was used as a standard. Diethyl ether (Junsei Chem. Co., Tokyo, Japan) was used for the anesthetization of rats. Standard buffers (Corning Med. and Sci. Halstead, Essex, U.K.) of pH 4.0 and pH 7.0 were used to calibrate the pH-meter. Lactic and butyric acids were purchased from Yakuri Pure Chem. Co., Ltd., Osaka, Japan. Deionized-distilled water (Milli-Q, Millipore Corp., Bedford, MA, U.S.A.) was used for the preparation and analysis of samples. All other chemicals were of analytical reagent grade.

Acidity Determination in Standard HCl Solution

Hydrochloric acid was appropriately diluted for the purpose from 1.0 N of stock standard solution. It was standardized with potassium bicarbonate. The stock solution of FCH was dissolved in acetone to the concentration of 1.0 mg/ml. Various volumes of HCl stock solution ranged from 1 μl to 1.0 ml were transferred into small test tubes. Acetone (1 ml) was added to each test tube and the appropriate volume of deionized-distilled water was added to make up 2.0 ml. FCH stock solution (2.0 ml) was appended to each test tube immediately before the fluorescence measurement. The total volume of each sample was 4.0 ml. Each determination was quintuplicated.

The maximum wavelengths of excitation and emission in the spectro-fluorometer were fixed at 392.0 nm and 447.0 nm, respectively. Response time was 4 sec and the photomultiplier tube gain condition was adjusted to medium. The slit band widths of both excitation and emission were 3.0 nm.

Effect of lactic and butyric acids on gastric acidity

The effect of lactic and butyric acids which can coexist with gastric juice in the carcinoma of stomach and pyloric stenosis was considered. Various volumes, 1 μl to 700 μl, of 1.0 N stock solution of each organic acid were transferred into small test tubes and 100 μl of HCl standard solution was added. Acetone (1 ml) was appended to each sample and 2.0 ml of FCH stock solution was also added immediately before the measurement.

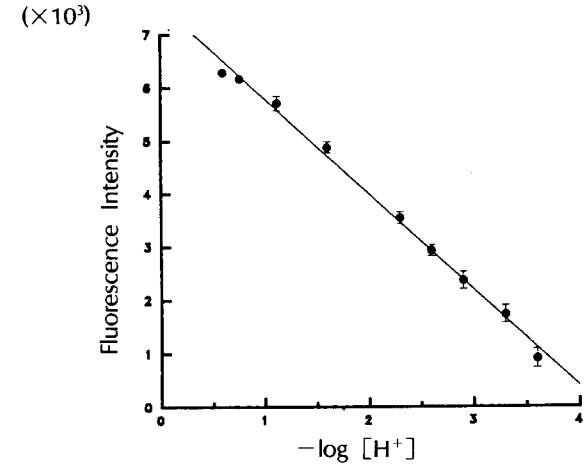


Fig. 1. Calibration curve of inorganic hydrochloric acid by the FCH method. The 1.0 N stock solution of hydrochloric acid was diluted to each pH sample solution in the range from pH 0.60 to pH 3.60. The intensity of fluorescence was measured at 447.0 nm with excitation at 392.0 nm. Linearity was gained from pH 0.60 (0.25 N) to pH 3.60 (0.00025 N). ($Y=-1646.1X+7059.5$, $\gamma=-0.996$)

Sample Preparation of Gastric Juice

Rats (Sprague-Dawley strain, 150-200 g, n=23) were fasted for 24 hr with free access to water. The rats were lightly anesthetized with diethyl ether and a 2 cm-midline incision was made. The duodenum was identified and the junction between the pylorus and the duodenum was picked up gently with a curved probe. The pylorus was ligated with surgical silk (No.4). After 7 hr following the ligation, the rat was sacrificed and the stomach was removed. A small incision was made in the stomach with a fine pair of scissors and gastric contents were collected (Hino *et al.*, 1989; Risley *et al.*, 1947). On the other hand, the gastric juice of humans (n=31) was collected from medical cases at the Chonnam National University Hospital.

Following sonication for 1 min, centrifugation was performed at 15,000×g for 5 min to remove regurgitated materials in the gastric juice.

pH Determination of Samples

The pH of rat and human gastric juice was measured by both pH-metry and the FCH method. A pH-meter was standardized by standard buffers with pH values of 4.0 and 7.0 prior to measurement. The electrode was thoroughly rinsed with distilled water and gently wiped between measurements. In the case of

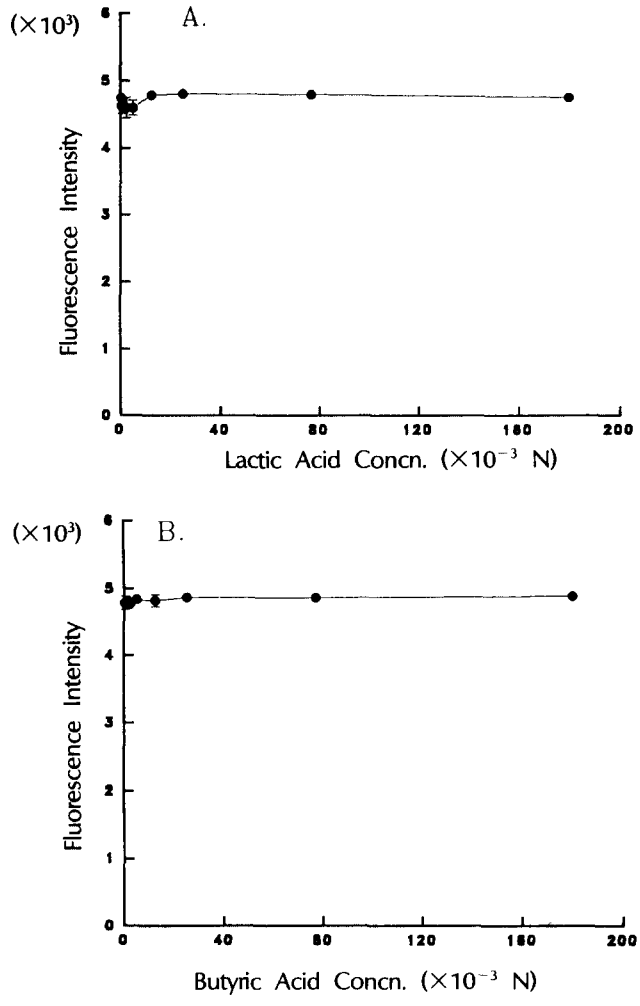


Fig. 2. Effects of lactic and butyric acids on the fluorescence intensity of FCH. Fluorescence intensities were not affected in the presence of lactic (A) and butyric (B) acids when HCl concentration was 0.025 N.

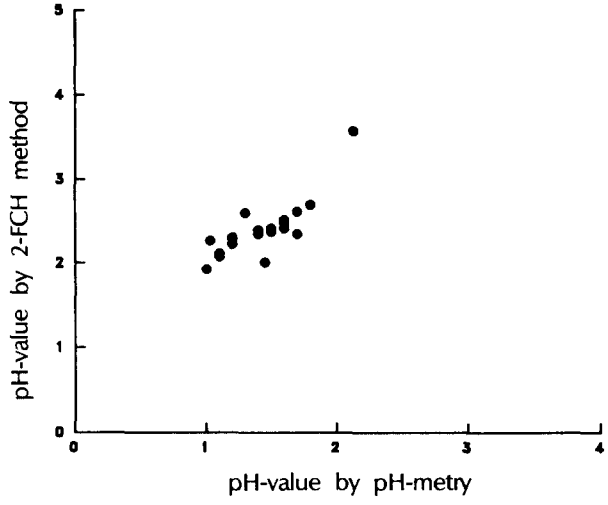


Fig. 3. The pH values of gastric juice of rats by pH-metry and FCH method plotted in X-Y style.

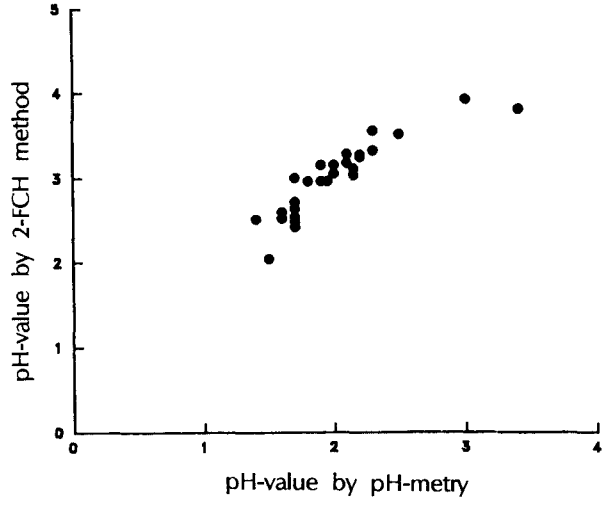


Fig. 4. The pH values of gastric juices of humans by the pH-metry and the FCH method plotted in X-Y style.

the FCH method, a standard addition method was used with 100 μl of gastric juice. Four portions of a given sample were prepared by using increased concentrations of HCl standard solutions. Acetone (1 ml) was added to the test tube and the appropriate volume of distilled water was added to make up 2.0 ml. A FCH stock solution (2 ml) was appended to the test tube immediately before the measurement. Fluorescence intensities obtained from each sample were plotted. Extrapolation of the line through the ordinate and back to the abscissa provided the pH of each sample. All pH measurements of samples were carried out in an air-conditioned room. Data obtained were statistically treated using the SPSS/PC$^+$ program.

RESULTS

Acidity Determination in Standard HCl Solution

The reactivity of FCH with inorganic hydrochloric acid made it possible to obtain a wide range linear relationship from pH 0.60 to pH 3.60 (0.25 N to 2.5×10^{-4} N). The correlation coefficient was -0.996 (Fig. 1).

Effect of Lactic and Butyric Acids

Lactic and butyric acids from 2.5×10^{-4} M to 1.8×10^{-1} M had no interference on the fluorescence intensity of FCH when they were present in the HCl standard solution (Fig. 2A, B).

Acidity Determination in Gastric Juice of Rats

The pH values obtained by the FCH method were higher (about 1 ± 0.3 unit) than those values obtained

by of pH-metry with 23 samples of rats. The correlation between the two methods appeared to be satisfactory with a correlation coefficient of 0.806 ($Y=0.934X+1.054$, Fig. 3) and they appear to be statistically significant ($P<0.001$).

Acidity Determination in Gastric Juice of Humans

The pH values obtained by the FCH method from the 31 human samples were higher about (1 ± 0.2 unit) in comparision with the values by pH-metry as seen in the case of rats. A linear relationship was found between the two methods ($Y=0.876X+1.25$, $\gamma=0.887$) as shown in Figure 4, and they showed significant correlation ($P<0.001$).

DISCUSSION

The differences in pH between pH-metry and the FCH method were approximately 1.0 ± 0.3 unit for most samples of rats and humans. The reason was not clear, however it was assumed that impurity quenching may be involved in the detection of fluorescence (Guilbault, 1973). The mean values of the differences were 0.96 and 1.01 in rats and humans, respectively. These can be utilized as correction factors. In fact, the values of pH-metry could also be obtained from the calculated pH values of the FCH method by subtracting the correction factor.

The FCH method would be a useful detection method of acidity at a low pH range. Moreover, the FCH method has an advantage over pH-metry in that it needs only a small amount of sample. Based on the experimental results, we can conclude that the FCH method is comparable to pH-metry for the assay of gastric acidity.

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