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Assay of fish freshness using trimethylamine vapor probe based on a sensitive membrane on piezoelectric quartz crystal

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Abstract

A trimethylamine (TMA) vapor probe based on the sensitive membrane of chitosan, which encapsulated pimelic acid on piezoelectric quartz crystal has been prepared for the assay of fish freshness. The response of the probe to TMA is rapid and completely reversible at normal temperature and relatively high humidity. The probe has an exponential response to TMA in the concentration range of 5–200 ppm, and the relative standard deviations (R.S.D.) were less than 5% for repeated determinations of 50 ppm TMA within 6 months. The results of fish freshness assay were consistent with those obtained by other methods and the sensory test.

Keywords: Trimethylamine vapor probe; Piezoelectric quartz crystal; Fish freshness; Chitosan

1. Introduction

The assay of fish quality and freshness is an important task for both the consumer and food industry. Odor is usually utilized to discriminate fish freshness in daily life, but total volatile base nitrogen (TVB-N) is still widely used index. A *K* value calculated from the results of quantitative analysis of adenosine triphosphate related compounds in fish muscle successfully determined the fish freshness. However, TVB-N or *K* value methods are rather time-consuming and require pretreatment of fish samples. Concentrations of trimethylamine (TMA) alone are also proposed as relevant parameters because TMA is a volatile amine produced immediately after the death of fish and its concentration increases with the lowering of the fish freshness. So, TMA vapor is more suitable to measure fish freshness with no damage and field applications. The semi-conducting metal oxide sensors [1,2] are the current research in this context part. However, high operating temperature is necessary for these sensors. A highly sensitive sensor that does not need heating apparatus is more adaptable for real-time and on-line fish freshness assay.

Piezoelectric crystal and the thickness-shear-mode (TSM) device have been used as chemical sensors in the gas phase due to its high mass-sensitivity. Guilbault et al. have developed TSM acoustic wave devices in recent years, and quartz crystals coated with various membranes have already been applied to detect a lot of analytes such as air pollutants [3,4], polar organic vapor [5], carbon dioxide [6], and biological substances [7]. Chitosan, a polymer prepared by deacetylation of natural chitin, is an interesting material for preparing sensitive membrane. One of the authors has previously reported an electrochemiluminescence sensor having a sensitive chitosan membrane for determinations of oxalic acid and amino acids [8]. In this work, we used chitosan for encapsulating pimelic acid and immobilized it on piezoelectric crystal, preparing TMA vapor probe for assay of fish freshness.

2. Experimental

An At-cut piezoelectric crystal of 9 mm in diameter (TSM) having silver-plated electrodes of 5 mm in diameter on each side with a fundamental frequency of about 8 MHz (Yashuda Electric Co., Shenzhen, China) was employed as the transducer of the probe. After both surfaces of the electrode were coated with adsorptive material, the probe was placed in a sealed chamber of volume of 2.16 dm^3. The probe and a monolithic microcomputer constituted an oscillation circuit, which was driven by a 5 V dc voltage and gave a stable frequency output. The frequency changes were measured with an 8-figure digital frequency counter

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(HC-F1000L, Zhonghuan Radio Co., Tianjin, China) or a 500 MHz oscilloscope (LT322, LeCroy Co., China). The relative humidity was monitored by a humidometer, which has been standardized by the aid of a series of salt saturated solutions. The working temperature of the sensor was adjusted by a water thermostat, but in normal experiments it was kept at room temperature (20 °C). The experiment of gas chromatography was performed by a HP-6890 gas chromatograph (HP Co., USA; capillary column, HP-35; detector, FID). The determination of TVB-N was operated in accordance with state standard (GB 5009, 44-85) for China.

Chitosan (95% deacetylation, Katokichi Co., Kagawa, Japan) was further deacetylated by heating in a 40% aqueous sodium hydroxide in an atmosphere of Ar. The deacetylation degree of the chitosan obtained was estimated to be above 99% by the inspection of its ^{1}H-NMR spectrum in 2% CD_3COOD/D_2O. The standard samples of TMA, CO_2, SO_2, H_2S, CO, NH_3 were purchased from Dalian Guangming Institute of Chemical Engineering. The gases of low concentration were obtained from dilution of the standard gases by air made from highly purified N_2 and O_2. Pimelic acid and other chemicals were of analytical grade or better.

The membrane coating on the electrode was prepared from a 1 ml 3% acetic aqueous solution contained 5 mg/ml chitosan and 3 mg/ml pimelic acid. The precursor solution was brush-coated in a controlled way over the surface of the electrodes. This process was repeated for several times until the coated amount, ΔM_m, equals to the desired quantity deduced from the frequency change, Δf_m. The relationship between Δf_m and ΔM_m is given by $\Delta f_m = -1\,\text{Hz} \cong \Delta M_m = 8.62\,\text{ng/cm}^2$.

3. Results and discussions

3.1. Response of the probe to TMA vapor

Typical responses (Δf) of the probe coated with pimelic acid/chitosan membrane to TMA vapor are shown in Fig. 1. After the probe is exposed to TMA vapor, which results in frequency shift, next the frequency changes quickly. Then this change decreases slowly, and at last, Δf reaches a steady value within 2 min. As the probe ceases to be in contact with TMA vapor, the frequency immediately returns to the original value within 1 min, and this implies that the responses are completely reversible. The time-dependent probe signal, Δf, increases with the increase of TMA concentration in the range of 5–250 ppm. Principles related to the response of TSM acoustic mode in the gas phase have been previously reviewed [9]. For At-cut piezoelectric crystals, if material is deposited or adsorbed on the surface of the crystal, an increase in the oscillating mass ΔM (g) results with the change of resonant frequency, Δf (Hz), as described by

$$\Delta f = -C_f \left(\frac{f_0^2}{A}\right) \Delta M \tag{1}$$

where A is the surface area in cm^2, C_f the mass-sensitivity constant ($2.26 \times 10^6\ \text{cm}^2\ \text{s/g}$), and f_0 the fundamental frequency (MHz). In our experiments, it has been found that Δf is directly proportional to the mass of chitosan encapsulated pimelic acid membrane on the electrodes: $\Delta f(\text{Hz}) = -0.116\Delta M\ (\text{cm}^2/\text{ng})$ (correlation coefficient $= 0.994$). This result is in good agreement with the prediction by Eq. (1).

However, linear response of the probe to TMA does not exist in a wider concentration range. As shown in Fig. 2, a nearly straight line is only observed in a narrow range of 10–100 ppm, but as a whole, the response profile displayed an exponential increase with increasing TMA concentration. This result may be explained by the following consideration. As we know, coating on the surface of electrode with precursor solution is due to the mass deposition, but the deposition of TMA vapor onto the membrane is an adsorption process. In the latter case, the adsorbed amount of the gas on the surface may be described by Freundlich equation

$$M = kp^{1/m} \tag{2}$$

where M is the amount of the adsorbed gas on the solid surface (g), p the equilibrium pressure of the gas, m is an

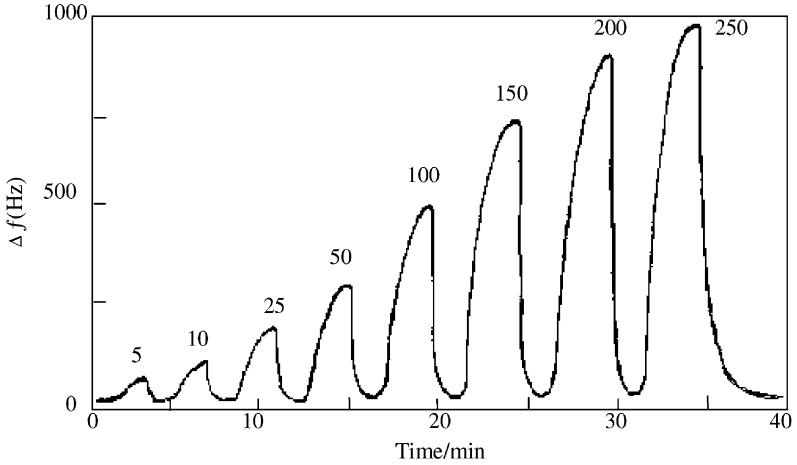

Fig. 1. Frequency change Δf of TMA probe as a function of time to different concentrations of TMA (numerals in the figure, ppm).

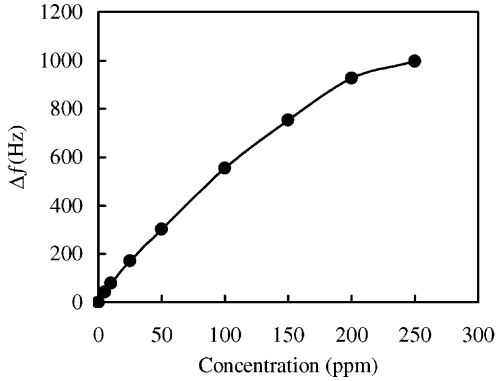

Fig. 2. Equilibrium values of the frequency Δf vs. TMA concentration.

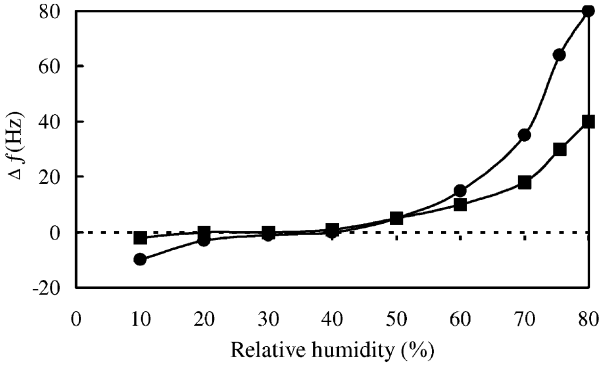

Fig. 4. Equilibrium values of frequency shifts Δf vs. relative humidity (●: without TMA; ■: in the presence of 50 ppm TMA).

empirical factor, and k is a constant at the specified temperature. Combining Eqs. (1) and (2), frequency change, Δf, in the adsorption process may be given by

$$\Delta f = -C_f \left(\frac{f_0^2}{A}\right) k p^{1/m} \tag{3}$$

Under conditions of low pressure and normal temperature, TMA vapor can be regarded as an ideal gas. Thus, from Eq. (3) we have

$$\Delta_f = -C_f \left(\frac{f_0^2}{A}\right) k' C^{1/m} = K C^{1/m} \tag{4}$$

This may also be expressed in the logarithm form

$$\log \Delta f = \log K + \frac{1}{m} \log C \tag{5}$$

where k' and K are constants at a given temperature. Fig. 3 shows a linear calibration curve for $\log \Delta f$ versus $\log C$ in the concentration range of 5–200 ppm TMA.

3.2. Effect of humidity

Because of the acid–base and/or the hydrogen bond interaction between the membrane and the adsorbates, TMA response might be affected by the presence of water vapor (humidity). We have tested the frequency shift of the probe in the range of 10–80% relative humidity as TMA is present or absent in the system. If the equilibrium value of Δf in 40% relative humidity is used as the relative standard, typical results are shown Fig. 4. It can be seen that as TMA is not present and the relative humidity is ranging from 10 to 60%, the response of the probe is relatively low, but it increases rapidly when the relative humidity exceeds 70%. In the presence of 50 ppm of TMA, however, the response of the probe to the humidity is much lower than in the former case. This may be due to the stronger competition of adsorption of TMA on the membrane. It seems that for relative humidity from 10 to 60%, its effect on TMA determination is not significant. When the relative humidity is higher than 70%, a remarkable effect on TMA results, but this interference can be corrected by using a humidity compensation device.

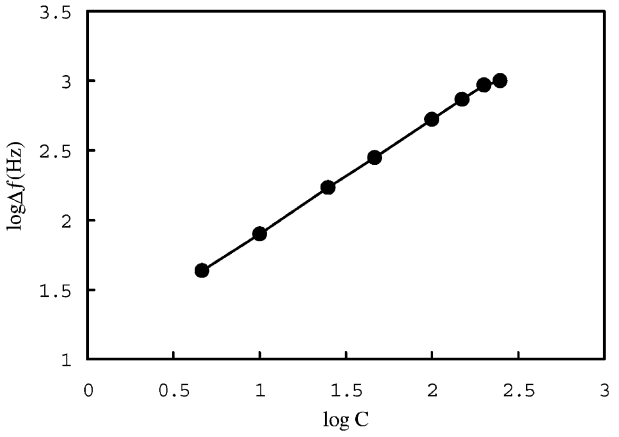

Fig. 3. Logarithm values of Δf vs. TMA concentration (calculated from Fig. 2).

3.3. Effect of temperature

For At-cut piezoelectric crystal, the temperature in principle does not affect the oscillation since its temperature coefficient is zero. But temperature may affect the chemical adsorption of TMA onto the membrane. We monitored the responses of the probe to 50 ppm TMA at different temperatures, from 10 to 50 °C, as shown in Fig. 5. It can be seen that the probe response decreases with increasing temperature, and then large temperature difference is not beneficial for the determination of TMA. As compared with air, it is clear that the sorption effect and chemical reaction are affected by temperature but not the membrane itself.

3.4. Effect of interferences by other gases

We have investigated the interference of other gases, such as CO_2, SO_2, CO, NO_2, H_2S and NH_3 on TMA measurements. Results obtained indicate that common amount of CO_2 in air, 1000 ppm of SO_2 and CO, 500 ppm of H_2S, 300 ppm of NO_2 do not affect TMA detection. TMA probe also responded to NH_3, dimethylamine and histamine vapor,

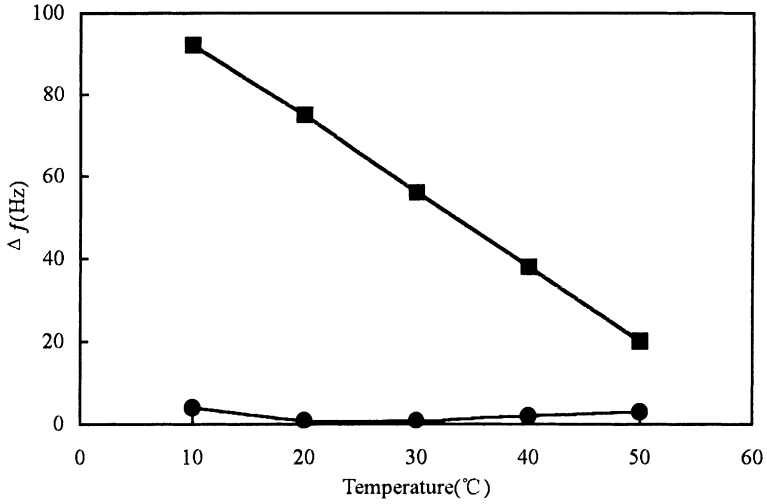

Fig. 5. Frequency changes as a function of temperature for the probe in 50 ppm TMA (■) and air (●).

and they could interfere with the determination of TMA. But for the measurement of fish freshness, these effects are negligible because in the initial putrid stage very small amounts of NH_3 and other amine may be produced after the death of the fish.

3.5. Durability and repeatability of the probe

To meet the requirement of practical application, TMA probe must have a longer longevity and higher repeatability. Chitosan, a polyaminosaccharide prepared by deacetylation of chitin, is a very stable material. Pimelic acid encapsulated by chitosan was immobilized on the surface of the crystal with the aid of its high viscosity, and thus, the property of the functional material can be maintained. Table 1 shows the long-time stability of the sensitive membrane. The response to 50 ppm of TMA was measured at different times within 6 months, and Δf stabilized at about 310 Hz. The relative standard deviations (R.S.D.s) were less than 5% for five repeated determinations in every measurement.

Table 1
Stability of TMA probe

Time	1 day	1 week	1 month	2 months	4 months	6 months
Δf (Hz)	296	305	302	308	312	314
R.S.D. (%)	6.1	4.9	4.6	4.5	4.2	5.2

3.6. Determinations for actual sample

To test the possibility of practical application of this method, we used TMA probe to monitor the freshness of carp by determination of TMA concentration after the death of the fish. Simultaneously, TVB-N and GC methods were

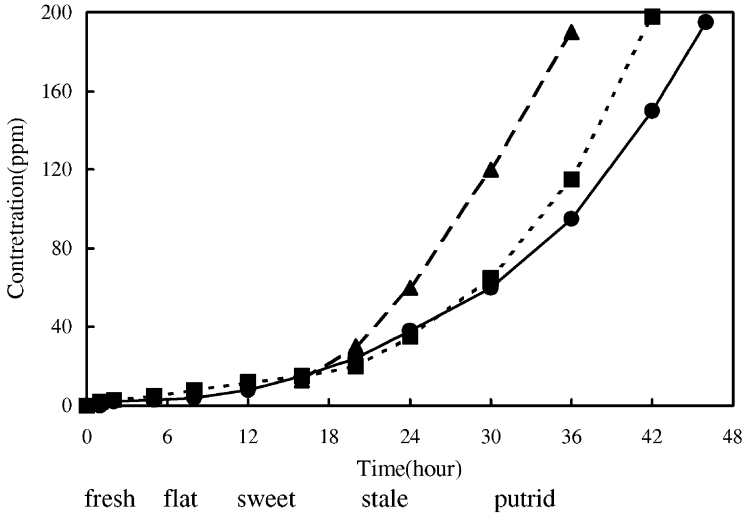

Fig. 6. Relationship between TMA concentration and the time after death of the fish, as well as fish freshness (human sensation). TMA concentrations were determined by TMA probe (●), TVB-N method (▲) and GC (■), respectively.

applied for comparison. As shown in Fig. 6, freshness of the fish monitored by TMA probe was consistent with TVB-N and GC methods as well as the sensory test.

4. Conclusions

TMA vapor probe based on sensitive membrane coated with chitosan encapsulated pimelic acid on piezoelectric crystal shows good sensitivity, high selectivity and high level of reversibility and reproducibility in the continuous monitoring of fish freshness. The response of the probe towards TMA is based on acid–base reaction. This probe element shows principal advantages because of its easy handling, low costs and fast response. Especially, the probe can be operated at normal temperature and relative high humidity, and may be conveniently applied in the assay of fish freshness.

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Biography

Changzhi Zhao is an assistant professor at Dalian University of Technology. He graduated in chemistry from Liaoning Normal University. He received MS degree in instrumental analysis from Dalian University of Technology in 1989, and PhD in functional material chemistry from Oita University (Japan) in 1998. His current research interests are chemical sensors.