Determination of Ethanol in Sake Mash by a Chloride Ion-Selective Electrode

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A chloride ion-selective solvent polymeric membrane electrode (Cl-ISE) using quaternary ammonium salts for determination of the ethanol concentration during sake fermentation is described. The ethanol response by a Cl-ISE is based on the change in the transfer activity coefficient of Cl⁻ from water to ethanol. The logarithm value of the transfer activity coefficient increases linearly with increasing ethanol concentration. The Cl-ISE using a mixture of *o*-nitro-phenyloctyl ether and *n*-tetradecylalcohol as plasticizers and tetradodecylammonium salts as sensing materials showed good linearities for ethanol (−2.39 mV/%) and for Cl⁻ (−53.6 mV/decade). Furthermore, the interference from co-existent components in sake mash with the response of the Cl-ISE was improved in comparison with that using other plasticizers and sensing materials. The Cl-ISE was found to show good accuracy in the measurement of ethanol in sake mash.

Keywords: ethanol, chloride ion-selective solvent polymeric membrane electrode, transfer activity coefficient, quaternary ammonium salts, sake mash

As is well-known, the ethanol concentration during sake fermentation is one of the most important pieces of information to maintain its quality. In order to measure the ethanol concentration in sake mash, a number of on-line sensing systems have been proposed (Samuta *et al.*, 1992; Kondo *et al.*, 1986; Oishi *et al.*, 1990). However, those systems require large expensive devices, such as a gas sensor, biosensor, gas chromatograph, special filters, etc. In addition, these devices always require complicated maintenance. To overcome these problems and correctly measure the ethanol concentration in sake mash, a simple alternative method would be advantageous.

We have previously reported that the response of a chloride ion-selective solvent polymeric membrane electrode (Cl-ISE) using quaternary ammonium salts to ethanol would be based on the change in the transfer activity coefficient of Cl⁻ from water to ethanol. In Cl-ISEs, for a given concentration of Cl⁻, the electrical potential decreases linearly with an increase in the concentration of ethanol (Tsukatani & Toko, 1998; Arikawa *et al.*, 1996). This result suggested that the Cl-ISE could be utilized to measure the ethanol concentration. However, the Cl-ISE had a low selectivity for co-existent components in sake mash. Shibata *et al.* (1992, 1993) have reported that aliphatic alcohol compounds as plasticizers and tetraalkylammonium salts with long alkyl groups as sensing materials were more effective for improving the selectivity of Cl-ISEs. In this paper, the effects of plasticizers and sensing materials on the ethanol response in addition to the selectivity were studied in order to develop a simple method that is capable of measuring the ethanol concentration during sake fermentation.

Theoretical

The electrical potential of a Cl-ISE using quaternary ammonium salts in KCl solution is expressed by

$$E = S_{\text{Nernst}} \log \gamma_{\text{Cl}^-} + S_{\text{Nernst}} \log [\text{Cl}^-] + E_0 \quad (1)$$

where γ_{Cl^-} , $[\text{Cl}^-]$ and S_{Nernst} are the activity coefficient, the concentration (mM) of Cl⁻ and the Nernstean slope (mV/decade), respectively. In binary ethanol-water mixtures, the activity coefficient of Cl⁻ would change according to the mixture ratio. The logarithm value of the activity coefficient is known to increase linearly with increasing ethanol concentration (Marcus, 1985). From the previous report (Tsukatani & Toko, 1998), the electrical potential relative to ethanol concentration would be given by

$$\Delta E = E - E_0 = S_{\text{Nernst}} \log \gamma_{\text{Cl}^-} (\text{Cl}^-, \text{water} \rightarrow \text{ethanol}) \times 1/100 \\ \times [\text{ethanol}] + S_{\text{Nernst}} \log \gamma_{\text{Cl}^-} (\text{ethanol solution}) \\ + S_{\text{Nernst}} \log [\text{Cl}^-] \quad (2)$$

where $\gamma_{\text{Cl}^-} (\text{Cl}^-, \text{water} \rightarrow \text{ethanol})$ represents the transfer activity coefficient of Cl⁻ from water to ethanol. $\gamma_{\text{Cl}^-} (\text{ethanol solution})$, which depends only on the concentration of Cl⁻, is given by the Debye-Hückel equation (Okazaki & Sakamoto, 1990). $[\text{ethanol}]$ is the ethanol concentration (%). Because the change in $S_{\text{Nernst}} \log \gamma_{\text{Cl}^-} (\text{ethanol solution})$ might be much smaller than that of the first term in Eq. (2) in the range of ethanol from 0 to 20%, the second term could be ignored. Therefore, Eq. (2) can be expressed by

$$\Delta E = S_{\text{Nernst}} \log \gamma_{\text{Cl}^-} (\text{Cl}^-, \text{water} \rightarrow \text{ethanol}) \times 1/100 \\ \times [\text{ethanol}] + S_{\text{Nernst}} \log [\text{Cl}^-] = S_{\text{Transfer}} [\text{ethanol}] \\ + S_{\text{Nernst}} \log [\text{Cl}^-] \quad (3)$$

where the slope for $[\text{ethanol}]$ is defined as

Fig. 1. Response of the Cl-ISE to ethanol and Cl⁻. The polymeric membrane contains NPOE as plasticizers and trioctylmethylammonium salts as sensing materials. KCl: (□) 0.1, (■) 0.3, (◇) 1.0, (◆) 3.0, (○) 10, (●) 30, (△) 100 mM.

$$S_{\text{transfer}} \equiv S_{\text{Nernst}} \log \gamma t (\text{Cl}^-, \text{water} \rightarrow \text{ethanol}) \times 1/100$$

Figure 1 shows the electrical potential of a Cl-ISE using quaternary ammonium salts relative to the ethanol concentration. The electrical potential fell linearly with increasing ethanol concentration, and all slopes of the response lines were equal in different KCl solutions from 0.1 to 100 mM. This means that the electrical potentials of the Cl-ISE depend on the concentrations of ethanol and Cl⁻ as shown in Eq. (3).

If the concentration of Cl⁻, which is one of the major anions contained in sake mash, is constant, the change in the electrical potential in Eq. (3) will depend only on the ethanol concentration. Due to the fact that the concentration of Cl⁻ is almost constant during sake fermentation, the second term in Eq. (3) would be constant. S_{transfer} and S_{Nernst} are determined using known standard solutions. Hence, once the value of the second term is calculated from the values of the initial ethanol concentration measured by other methods, such as gas chromatography, and the electrical potential obtained by the Cl-ISE, the ethanol concentration can be estimated from only the electrical potential in subsequent measurements.

Materials and Methods

Reagents Polyvinyl chloride was obtained from Wako Pure Chemical Industries (Osaka). 2-Fluoro-2'-nitro-diphenyl ether and *o*-nitro-phenyloctyl ether (NPOE) were obtained from Dojindo Laboratories (Kumamoto). Diocetylphenyl phosphonate was purchased from Aldrich Chemical Co. (Milwaukee, WI). Dioctyl adipate, dioctyl phthalate, *n*-decylalcohol, *n*-dodecylalcohol, *n*-tetradecylalcohol, *n*-octadecylalcohol, trioctylmethylammonium salt and dioctadecyldimethylammonium salt were bought from Tokyo Kasei (Tokyo). Tridodecylmethylammonium salt, trioctadecylmethylammonium salt and tetradodecylammonium salt

were purchased from Fluka (Buchs, Swiss). All other chemicals were of analytical reagent grade and were used without further purification.

Membrane and cell assembly The polymeric membranes were prepared as described previously (Oohira & Toko, 1997). Five kinds of sensing materials and 6 kinds of plasticizers were utilized for the polymeric membranes; each sensing material was mixed with 800 mg polyvinyl chloride and 1.0 ml plasticizer, which were dissolved in 18 ml tetrahydrofuran and dried on a glass plate. The polymeric membrane thus prepared was a transparent soft film about 0.2 mm thick. The polymeric membrane was then cut into small disks and mounted on a hollow-cylinder electrode. The cell assembly for the potentiometric measurement was as follows:

Ag/AgCl|3 M KCl||salt bridge (agar)||sample solution|polymeric membrane|3 M KCl|Ag/AgCl

The salt bridge of the reference electrode contains 3 M KCl and agar. The Cl-ISE was made of an Ag wire whose surface was plated with Ag/AgCl, with an internal cavity filled with 3 M KCl solution.

Measuring apparatus A Cl-ISE, an Ag/AgCl reference electrode (HS-205C, TOA Electronics, Ltd., Tokyo) with a salt bridge, and an electrometer (R8240, Advantest, Tokyo) were used for all potentiometric measurements. The electrical potential difference between a Cl-ISE and the reference electrode was measured. The electrical potentials were determined at a temperature of 22 ± 1°C. Each Cl-ISE was pre-conditioned with 100 mM KCl solution overnight before the measurements.

Measurement of sake mash To prevent the influence of solid substances on the polymeric membrane in the application of the Cl-ISE to sake mash, the tips of the Cl-ISE and the reference electrode were fitted with stainless steel mesh tubes. In addition, vibration was applied to both electrodes in order to promote the diffusion of sake solution onto the membrane surface for 10 s before starting the measurement. The stabilizing times of the electrical potential were 30 s until its change became within ± 0.1 mV. The measurement was accomplished at a temperature of 15 ± 1°C.

Analytical method The ethanol concentration was measured by a gas chromatograph (Model GC-6A, Shimadzu Corp., Tokyo). The titrable acidity and the amino acidity were determined by the official method of analysis of the National Tax Administration Agency of Japan.

Results and Discussion

Plasticizers The effect of plasticizers on the response of Cl-ISEs using quaternary ammonium salts was studied. Trioctylmethylammonium salts were used as sensing materials and ether, ester and alcohol compounds were used as plasticizers. Figure 2 (a) and (b) show the effect of plasticizers on the responses to ethanol (0–20%) and Cl⁻ (0.1–100 mM), respectively. Some Cl-ISEs showed good Nernstean slopes of –50.3 to –54.0 mV/decade for Cl⁻, and others had slopes that were less than –50 mV/decade. However, all had good linearities for ethanol with slopes of –1.03 to –2.13 mV/% at constant KCl concentration (1.0 mM). When NPOE was used as the plasticizer, the maximum responses to ethanol and Cl⁻

Fig. 2. Effect of plasticizers on the response of the Cl-ISE. (a) Response to ethanol. (b) Response to Cl⁻. (c) Influence of co-existent components. Plasticizer: ($\square$) 2-Fluoro-2'-nitrodiphenyl ether, ($\blacksquare$) *o*-nitro-phenyloctyl ether, ($\diamond$) dioctyl adipate, ($\blacklozenge$) dioctyl phthalate, ($\circ$) dioctylphenyl phosphonate, ($\bullet$) *n*-decylalcohol.

were observed. In general, it is thought that the membrane electrode with a solvent having a relatively high dielectric constant such as NPOE has high quantitative extractability, because ion-exchangers and counter ions almost dissociate in the membrane. Hence, the high responses to Cl⁻ and ethanol were observed when NPOE was used as the plasticizer.

Sake mash contains a number of anions such as organic acids and amino acids. Those components could affect the responses of Cl-ISEs based on the ion-exchange reaction by quaternary ammonium salts (Matsui & Freiser, 1970; Shibata *et al.*, 1994). Thus, the influence of co-existent components in sake mash on the responses of Cl-ISEs was investigated. The mixtures (Table 1), which contained 3 kinds of organic acids (succinic acid, lactic acid and malic acid) and 14 kinds of amino acids (glycine, alanine, valine, leucine, isoleucine, phenylalanine, proline, serine, threonine, cysteine, aspartic acid, glutamic acid, lysine and arginine) at different concentrations, were utilized in this experiment. Those mixtures can be considered synthetic sake mash (Nakagawa *et al.*, 1996). Each synthetic sake mash contains organic acids and amino acids at the concentrations corresponding to the titrable acidity and the amino acidity in Table 1, respectively. As is shown in Fig. 2 (c), the electrical potential decreased with increasing titrable acidity of the synthetic sake mash. Likewise, the increase in the amino acidity of synthetic sake mash also caused a decrease in the electrical potential. Furthermore, the interference from co-existent components was shown to differ from one plasticizer to another. The Cl-ISE containing *n*-decylalcohol had the best result relative to the interference but showed the minimum response to ethanol. Shibata *et al.* (1993) have reported that aliphatic alcohol compounds were more effective as plasticizers in order to improve the selectivity of the Cl-ISEs. Our experiments also gave the same results as shown in Fig. 2 (c). Because the extent of the ion pair dissociation is small enough to neglect in the membrane with *n*-decylalcohol having a relatively low dielectric constant, the quantitative extractability is low. Moreover, the decrease in dielectric constant of the solvent will not contribute to the specific extraction but can diminish the quantitative extractability of all ions. Therefore, the minimum response to co-existent components was observed when *n*-decylalcohol was used as the plasticizer. On the other hand, the Cl-ISE using NPOE was by far superior to the other electrodes from the point of view of ethanol response.

Table 1. Titrable acidity and amino acidity of synthetic sake mash.

	Titrable acidity ^{a)}	Amino acidity ^{b)}	KCl (mM)
No. 1	0.00	0.00	5.0
2	0.21	0.10	5.0
3	0.43	0.26	5.0
4	0.86	0.55	5.0
5	1.76	1.10	5.0
6	2.72	1.68	5.0
7	3.61	2.23	5.0
8	4.55	2.56	5.0

^{a)}Organic acids: succinic acid, lactic acid and malic acid.

^{b)}Amino acids: glycine, alanine, valine, leucine, isoleucine, phenylalanine, proline, serine, threonine, cysteine, aspartic acid, glutamic acid, lysine and arginine.

Thus, polymeric membranes using a mixture of NPOE and aliphatic alcohol compounds such as *n*-decylalcohol, *n*-dodecylalcohol, *n*-tetradecylalcohol and *n*-octadecylalcohol at a ratio of 9 : 1 were prepared. The effect of plasticizers on the interference from co-existent components in sake mash was then studied. The interference was expressed as the ratio of the response to synthetic sake mash (No.8) versus that to ethanol (20%). The smaller the ratio, the smaller the interference with the CI-ISEs. The results are shown in Table 2. Addition of aliphatic alcohol compounds to NPOE hardly changed the response to ethanol but remarkably diminished the response to synthetic sake mash (No. 8) except for *n*-octadecylalcohol. The point to observe is that the degree of the decrease in the response to synthetic sake mash (No. 8) was large compared with the decrease in the response to ethanol when a mixture of NPOE and *n*-tetradecylalcohol was used as the plasticizer. This means that addition of *n*-tetradecylalcohol to NPOE is most effective considering both the ethanol response and the interference. One explanation for this result may be that hydrogen bonds by a protic solvent such as *n*-tetradecylalcohol to anions affect the ion-exchange reaction of the CI-ISE because the protic solvent can solvate the anions more strongly than an aprotic solvent such as NPOE. Therefore, we selected the mixture of NPOE and *n*-tetradecylalcohol as the plasticizer.

Sensing materials In order to improve the ethanol response of CI-ISEs and reduce the interference from co-existent components, we attempted to use 5 kinds of quaternary ammonium salts with alkyl groups having 8–18 carbon

atoms as sensing materials with a mixture of NPOE and *n*-tetradecylalcohol as the plasticizer. All CI-ISEs showed good linearities for ethanol (−1.31 to −2.39 mV/%) and for Cl[−] (−51.4 to −54.2 mV/decade). As shown in Table 3, the maximum response to ethanol and the minimum interference were achieved when tetradodecylammonium salts were utilized as sensing materials. Shibata *et al.* (1992) have shown that the selectivity of CI-ISEs depends on the number of long alkyl groups and the alkyl chain length of the quaternary ammonium salts. When tetraalkylammonium salts with long alkyl groups having 14 or more carbon atoms were used as sensing materials, the selectivity for organic anions was improved compared with other quaternary ammonium salts. They have concluded, from what has been said above, that the molecular structure of quaternary ammonium salts with alkyl groups might affect the selectivity of CI-ISEs. However, there is not much evidence to prove that. Our results also showed that quaternary ammonium salts with 2 or 3 long alkyl groups having 18 carbon atoms were more effective as sensing materials with regard to responses to synthetic sake mash (No. 8). However, the CI-ISEs using these quaternary ammonium salts were by far inferior to the other electrodes from the point of view of ethanol response. As Table 3 indicates, the response to synthetic sake mash (No. 8) tends to increase with increasing response to ethanol. The important point to note is that the degree of the increase in the response to synthetic sake mash (No. 8) was small compared with the increase in the response to ethanol when tetradodecylammonium salts were used as sensing materials. This cannot be proved, but it seems likely that the molecular structure of tetradodecylammonium salts affects both the quantitative and specific extractability. Thus, tetradodecylammonium salts as sensing materials were used in the subsequent work.

Changes in the activity coefficient of other anions The CI-ISE responded to the change in the transfer activity coefficient of Cl[−] from water to ethanol. In fact, there are numbers of anions such as organic acids and amino acids in

Table 2. Effect of plasticizers on the response parameters.

	KCl response (mV/decade)	EtOH response (mV/%)	S.S. response ^{a)} (mV, 20%)	S.S. response ^{a)} (mV, No. 8)	Interference ^{b)}
NPOE	−54.0	−2.13	−43.2	−15.2	0.351
N:n-DA	−53.6	−2.09	−42.0	−11.5	0.273
N:n-dDA	−53.2	−2.05	−41.4	−10.8	0.261
N:n-tDA	−53.4	−2.11	−42.5	−10.8	0.254
N:n-oDA	−51.8	−1.84	−37.1	−9.5	0.256

Plasticizer: N, *o*-nitro-phenyloctyl ether (NPOE); *n*-DA, *n*-decylalcohol; *n*-dDA, *n*-dodecylalcohol; *n*-tDA, *n*-tetradecylalcohol; *n*-oDA, *n*-octadecylalcohol.

^{a)}S.S. represents the synthetic sake mash.

^{b)}Interference is expressed as the ratio of the response to synthetic sake mash (No. 8) versus that to ethanol (20%).

Table 3. Effect of sensing materials on the response parameters.

	KCl response (mV/decade)	EtOH response (mV/%)	S.S. response ^{a)} (mV, 20%)	S.S. response ^{a)} (mV, No. 8)	Interference ^{b)}
TOMA	−53.4	−2.11	−42.5	−10.8	0.254
TDMA	−54.2	−2.06	−41.2	−10.7	0.260
TODMA	−53.2	−1.31	−26.8	−7.5	0.280
TDA	−53.6	−2.39	−48.2	−11.3	0.234
DODMA	−51.4	−1.59	−32.0	−9.0	0.281

Sensing material: TOMA, trioctylmethylammonium salt; TDMA, tridodecylmethylammonium salt; TODMA, trioctadecylmethylammonium salt; TDA, tetradodecylammonium salt; DODMA, dioctadecyldimethylammonium salt.

^{a)}S.S. represents the synthetic sake mash.

^{b)}Interference is expressed as the ratio of the response to synthetic sake mash (No. 8) versus that to ethanol (20%).

Fig. 3. Correlation between the ethanol concentrations measured by the CI-ISE and by a gas chromatograph.

sake mash. Thus, changes in the activity coefficient of those anions by ethanol may occur and can affect the ethanol response of the Cl-ISE. We tried to investigate the response to 4 kinds of model sake solutions which were prepared as follows: each commercial sake was freeze-dried, and then the obtained powders were added to 0, 1, 3, 5, 10, 15 and 20% ethanol solutions, respectively. As shown in Table 3, the slope for ethanol solution containing 1.0 mM KCl was -2.39 mV/% with the Cl-ISE using the mixture of NPOE and *n*-tetradecylalcohol as plasticizer and tetradodecylammonium salts as sensing materials. On the other hand, slopes for model sake solutions were -2.37 , -2.37 , -2.38 and -2.42 mV/%, respectively. It follows from this that the Cl-ISE responds only to the change in transfer activity coefficient of Cl⁻ from water to ethanol and hardly responds to that of anions in sake.

Determination of ethanol concentration in sake mash

The Cl-ISE using the mixture of NPOE and *n*-tetradecylalcohol as plasticizer and tetradodecylammonium salts as sensing materials was used to determine the ethanol concentration in sake mash. The measurement was performed according to the method mentioned in the previous section. Figure 3 shows the results of the determination of ethanol in sake mash. The results obtained by the present method (y) were compared with those obtained by gas chromatography (x). A straight line, defined by the equation $y=0.945x+0.828$, with a standard deviation of the slope $S_m=0.030$ and of the intercept $S_b=0.410$, and a standard error of the estimate $S_{y,x}=0.545$, correlation coefficient $r=0.981$ ($n=42$), was obtained. The Cl-ISE was found to show good accuracy in the measurement of ethanol in sake mash.

Lifetime For a preliminary determination of the lifetime, the polymeric membrane electrode was stored in 20% ethanol solution containing 1.0 mM KCl when not in use. Over a month, the electrical potential as measured with 1.0 mM KCl without added ethanol gradually decreased by 10 mV (from 177.5 to 167.5 mV). On the other hand, response slopes for ethanol and Cl⁻ in linear ranges of the calibration curves did not change significantly (from -2.40 to -2.35 mV/% for ethanol, from -53.5 to -53.1 mV/decade for Cl⁻).

Conclusions

A new method for the determination of ethanol using a Cl-ISE has been developed. Compared to other methods used for the continuous analysis of ethanol in sake mash, this method could be performed more simply. When mixtures of NPOE and *n*-tetradecylalcohol as plasticizers and tetradode-

cylammonium salts as sensing materials were used, good results were obtained considering the ethanol response and the interference from co-existent components. Good correlation between the ethanol concentrations measured by the present Cl-ISE and by a gas chromatograph was observed ($r=0.981$) (Fig. 3). The interference from organic acids and amino acids could be ignored during sake fermentation. This result demonstrates the applicability of the Cl-ISE for the determination of ethanol in sake mash.

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